Universidade de Aveiro Departamento de Biologia

Ano 2017

Marcos Regueira Fernández

Ecologia do polvo-do-alto *Eledone cirrhosa* (Lamarck, 1798) no Atlántico Ibérico

The ecology of the horned octopus, *Eledone cirrhosa* (Lamarck, 1798) in Atlantic Iberian Waters

Ecología del pulpo blanco *Eledone cirrhosa* (Lamarck, 1798) en el Atlántico Ibérico

Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor (Programa Doutoral em Biologia; Ramo Biologia Marinha), realizada sob a orientação científica do Doutor Amadeu Mortágua Velho da Maia Soares, Professor Catedrático do Departamento de Biologia da Universidade de Aveiro, e do Doutor Ángel Francisco González González, Investigador Científico do grupo de Ecología y Biodiversidad Marina (ECOBIOMAR) do Instituto de Investigaciones Marinas de Vigo (IIM-CSIC).

Co Apoio financeiro da FCT através de uma Bolsa de Doutoramento atribuída a Marcos Regueira Fernández (SFRH/BD/51038/2010)

o júri

presidente

Doutor Delfim Fernando Marado Torres
Professor Catedrático, Departamento de Matemática da Universidade de Aveiro.

Doutor Ángel Guerra Sierra
Professor de Investigación de OPIs, Instituto de Investigaciones Marinas, C/ Eduardo Cabello S/N, CP 36208, Vigo (Pontevedra).

Doutor Fernando Manuel Raposo Morgado
Professor Associado com Agregação, Departamento de Biología & CESAM, Unilversidade de Aveiro.

Doutor José Vítor Sousa Vingada
Professor Auxiliar, Departamento de Biología, Universidade do Minho.

Doutor Álvaro Roura Labiaga
Investigador contratado. Instituto de Investigaciones Marinas, C/ Eduardo Cabello S/N. CP 36208, Vigo (Pontevedra).

Doutora Catarina Isabel da Costa Simões Eira
Equiparado a Investigador Auxiliar, Departamento de Biología & CESAM, Universidade de Aveiro

Doutor Ángel Francisco González González
Investigador Científico de OPIs, Instituto de Investigaciones Marinas, C/ Eduardo Cabello S/N, c.p 36208, Vigo (Pontevedra) (coorientador)

A mi padre

Agradecimentos

En primer lugar quiero agradecer al Departamento de Biología de la Universidade de Aveiro y al grupo de investigación de Ecología y Biodiversidad Marina (ECOBIOMAR) del Instituto de Investigaciones Marinas de Vigo (CSIC, Vigo), por acogerme y poner a mi disposición todos los recursos necesarios para la elaboración de éste trabajo. También a la Fundação para a Ciência e Tecnologia (FCT) por la financiación en forma de una beca de doctorado.

Agradezco a mis orientadores, los doctores Amadeu Soares y Ángel F. González, sus contribuciones para el correcto desarrollo de la fase de investigación, así como la concreción del presente documento. Quiero agradecer especialmente al profesor Ángel Guerra su apoyo y su ejercicio como orientador extraoficial de ésta tesis, a un nivel más allá de lo razonablemente esperable. Ha sido mucho lo que he podido aprender de él durante estos años y probablemente sin su apoyo éste trabajo no hubiera llegado a buen término.

Gracias a Marisa Ferreira y a Eugenio por su ayuda a la hora de conseguir los pulpos de Portugal y España, así como a la tripulación de *Nuevo Cigala*, que me consiguieron ejemplares vivos. También a las tripulaciones de los pesqueros *Vispón* y *Montefrisa Nueve*, con los que apredí de primera mano en que consiste el trabajo en el mar.

Quiero agradecer también al extenso listado de docentes, investigadores y compañeros con los que he tenido oportunidad de trabajar desde mi licenciatura. A los compañeros del Instituto Pirenaico de Ecología (CSIC, Jaca), y la gente con la que compartí aquella etapa de mi vida: Sergio, Guille, Arancha, Gonzalo, Popi, Emi, Darío, Juanillo y muchos más. También a la gente del Instituto de Ciencias del Mar (CSIC, Barcelona), y a mis profesores y compañeros de máster. Fue un autentico placer.

Gracias a la gente que ha contado conmigo en el plano laboral en el ámbito privado, que me ha permitido aprender mucho y de paso ganarme la vida dignamente haciendo lo que me gusta. Entre ellos Jose, Ángel y Ruth, Daniel, Ximo y Alberte.

Estoy muy agradecido a todos mis compañeros del Instituto de Investigaciones Marinas, y especialmente al personal de ECOBIOMAR: Alvarito, Jose, Garci, Mariana, Félix, Mac, María, Miguel, Helena, Fiona, Cuchi, Jaime, Jorge, Silvia, Lara, Santi… y mucha gente de prácticas, que han aportado su granito de arena para el buen ambiente reinante, no sólo en lo laboral, sino también en lo humano.

Si alguien merece una mención especial en éstos agradecimientos es Mª Teresa Fernández, mi madre y a la vez compañera de trabajo durante buena parte del desarrollo de ésta tesis. Una gran profesional, responsable, competente e incansable, a la vez que cariñosa y atenta, independientemente de las patadas que la vida te pueda propinar. Mamá, eres un modelo para mí, y me consta que también para muchas personas con las que has trabajado todos estos años. Ha sido una suerte y un placer que hayamos podido compartir esto.

Gracias a mis *mendrugos*, que siempre están ahí, sobre todo cuando hay churrasco de por medio. Varela, Iago, Uxío, Bea, Larri, Edu, Miguel, Senén y todos los demás. Que no me olvido, que es que no quiero eternizarme! Gracias chicos.

También mi agradecimiento a toda mi familia y en especial a Carli, al que espero ir a visitar poco después de la defensa de ésta tesis.

Por último a ti Edel, gracias por estar siempre ahí, comprenderme y saber aguantar y perdonarme mis cabreos, indecisiones, cansancios y ausencias.

Vaya desde aquí mi más sincero agradecimiento a todos ellos.

Palavras-chave

Cefalópodes, *Eledone cirrhosa*, ecologia, ciclo reprodutivo, crescimento, idade, distribuição, dieta, noroeste da Península Ibérica, Nordeste Atlântico.

Resumo

O presente trabalho aborda o estudo de diversos aspectos da ecologia do polvo-do-alto, *Eledone cirrhosa* (Lamark, 1798) na costa noroeste da Península Ibérica, entre o oeste do Golfo da Vizcaya ao norte de Portugal. O polvo- do-alto é um octópode bentónico que se distribuí ao longo da plataforma e do talude continental em toda a bacia mediterranica e do Atlántico nordeste, desde Marrocos até à Noruega, incluindo as Ilhas Britânicas e Islândia. No noroeste da Península Ibéria esta espécie é considerada acessória da pesca de arrasto, direccionada fundamentalmente à captura de pescada (*Merluccius merluccius*), tamboril (*Lophius sp.*) e o lagostim (*Nephrops norvegicus*). Os desembarques de polvo- do-alto mostram um padrão cíclico concordante com o ciclo de vida da espécie, embora o interesse comercial varie consideravelmente em toda a sua área de distribuição. De acordo com os relatórios do Conselho Internacional para a Exploração dos Mares (CIEM/ICES), os desembarques de *E. cirrhosa* nas subáreas VIIIc e IXa norte atingiram 1.145 t em 2013.

No que diz respeito ao tamanho (comprimento do manto, ML) máximo e parâmetros reprodutivos (maturação, fecundidade e o tamanho dos ovos / espermatóforos), os indivíduos das águas do noroeste da Península Ibérica têm características intermédias aos apresentados pelos indivíduos do norte da Europa e do Mediterrâneo, o que sugere um gradiente nas condições ambientais ao longo da sua área de distribuição. Este gradiente reflecte-se também nos resultados encontrados em nossa área de estudo.

Durante a amostragem realizada ao longo de dois anos, foi observada uma tendência significativa para uma proporção sexual, dominada pelas fêmeas. Na costa noroeste da Península Ibérica, o período de postura da espécie concentrou-se nos meses de maio e junho. De modo semelhante ao tamanho máximo, o tamanho da primeira maturação sexual aumentou com a latitude em ambos os sexos. No caso das fêmeas, o tamanho da primeira maturação variou entre 100 e 134 mm ML, e nos machos entre 91 e 108 mm ML, dependendo da zona de captura.

A análise das capturas por unidade de esforço (CPUE), obtidas em campanhas científicas feitas em diferentes momentos do ciclo reprodutivo da espécie, mostra variações na sua distribuição e abundância na costa noroeste da Península Ibérico. A modelação geoestatística das capturas descreve a migração para menores profundidades durante época de desova.

A dieta do polvo-do-alto no Atlântico Ibérico, foi estudada pela primeira vez através da combinação da análise visual e molecular do conteúdo do estômago. A dieta de *E. cirrhosa* nesta área é composta principalmente por crustáceos (74,64%), seguido de peixes (16,84%) e, em menor proporção, por equinodermes (5,51%), moluscos (4,92%) e poliquetas (2,09%). A dieta da espécie foi modelada por meio de regressão logística multinomial (MLR). De acordo com o modelo desenvolvido, os principais fatores que afetam aos padrões alimentares foram: o estado de maturação, os pesqueiros de origem e a época do ano. Os resultados, em combinação com a literatura existente sobre a distribuição das presas, sugerem que *E. cirrhosa* não é um caçador totalmente oportunista, mas sim um generalista selectivo.

No presente estudo estimou-se pela primeira vez a idade de *E.*

cirrhosa analisando os anéis de crescimento na concha vestigial (bastonete). Foi analisada uma sub-amostra de 122 indivíduos entre 67 e 950 g, seleccionados a partir de uma amostra total de 2220 indivíduos, através de análise de progressão modal (MPA) para representar todas as classes de tamanho. A idade estimada variou entre 106 e 516 dias e as fêmeas foram significativamente mais longevas (17 meses) do que os machos (14 meses). Com base nas idades estimadas a data de eclosão dos ovos foi calculada, concluindo que, embora existam nascimentos durante todo o ano, eles estão mais concentrados durante o inverno. A taxa de crescimento relativo instantânea, isto é, o aumento da percentagem de peso do corpo por dia, estimada para classes de idade de 90 dias, variou entre 0,03% e 2,17%, diminuindo ao longo do ciclo de vida dos indivíduos.

Fornece-se informação nova sobre o comportamento desta espécie, baseada na observação directa de exemplares no aquário. Alguns indivíduos iniciaram espontaneamente a postura em abrigos dispostos no interior dos aquários. Mais tarde verificou-se que, tal como no *Octopus vulgaris*, a espécie oferece cuidados maternais durante o período de desenvolvimento embrionário. Durante este período, as fêmeas ficam dentro do abrigo onde depositaram os ovos e concentram-se no seu cuidado. A espécie tolera as condições de cativeiro, indicando a sua aptidão para o cultivo tanto para fins experimentais como comerciais.

Apesar de sua importância para o sector das pescas, a escassez de estudos sobre *E. cirrhosa* faz com que a informação apresentada neste documento constitua um marco na compreensão do ciclo de vida da espécie na costa noroeste da Península Ibérica e, portanto, representa uma importante contribuição para o conhecimento global da teutofauna Ibérica.

Keywords Cephalopods, *Eledone cirrhosa*, ecology, reproductive cycle, growth, age, distribution, diet, NW Iberian peninsula, NE Atlantic.

Summary

This Ph.D. Dissertation deals on the study of several aspects of the ecology of the horned octopus *Eledone cirrhosa* (Lamark, 1798) in the NW Iberian Peninsula, from western gulf of Biscay to north Portugal. The horned octopus is a benthic octopod distributed along the continental shelf and slope throughout the Mediterranean and northeast Atlantic from Morocco to Norway, including the British Isles and Iceland. In the NW Iberian Peninsula this species is considered as a by-catch of trawling fishery, mainly targeting hake (*Merluccius merluccius*), monkfish (*Lophius* sp.) and Norway lobster (*Nephrops norvegicus*). Horned octopus landings show a cyclical pattern consistent with its life cycle, and its commercial interest considerably varies throughout its geographic range. According to ICES reports, landings of *E. cirrhosa* in VIIIc and IXa north subareas reached 1145 t in 2013.

In terms of both body size and main reproductive parameters (such as maturation, fecundity, and eggs/spermatophores size), specimens of *E. cirrhosa* from northwest Iberian Peninsula have intermediate characteristics to those exhibited by individuals from the north-eastern Atlantic Ocean and those from the Mediterranean Sea, suggesting some gradation on environmental conditions. This gradient, in turn, was also found in our study area.

During the sampling, carried out over two years, a significant bias was observed in the relative abundance between sexes, always favourable to females. The spawning season of the species also varies throughout its range. Specifically, in the northwest Iberian it concentrates in May and June. Similarly to the maximum size, the size-at-maturity increased with latitude. This population parameter ranged from 100 to 134 mm mantle length (ML) for females and between 91 and 108 mm ML for males, depending on the fishing ground.

The analysis of catches per unit effort (CPUE) obtained from scientific surveys and carried out at different times of the reproductive cycle of the species shows the existence of spatio-temporal variations on its distribution and abundance in the northwest Iberian sector. The geostatistical modelling of catches depicts the seasonal migration performed by mature individuals to shallower waters during the spawning season.

The diet of the horned octopus in the north-western Iberian Peninsula was studied for the first time by combining visual and molecular analysis of stomach contents. The diet of *E. cirrhosa* in the area was mainly composed by crustaceans (74.64%), followed by fish (16.84%) and, to a lesser extent echinoderms (5.51%), mollusks (4.92%) and polychaetes (2.09%). Multinomial logistic regression (MLR) was used for modelling feeding trends of the species. According to it, the main factors affecting feeding patterns were maturity stage, fishing ground and season. The obtained results, in combination with the previous literature on the distribution of prey, suggest that *E. cirrhosa* is not a totally opportunistic hunter, but rather a selective generalist.

The age of *E. cirrhosa* was estimated for the first time by stylet increment analysis. A total of 122 individuals, ranging from 67 to 950 g, were selected from a total sample of 2220 specimens using a modal progression analysis (MPA), in order to represent all size classes. The estimated age ranged from 106-516 days and females were

significantly more long-lived (17 months) than males (14 months). Based on the estimated ages, hatching date was back-calculated, concluding that hatchings, although occur throughout the year, are mostly concentrated during winter. The instantaneous relative growth rate, i.e. the increase in % body weight per day, estimated for age classes of 90 days, ranged between 0.03% and 2.17%, decreasing throughout the development of individuals.

New behavioural information on this species is provided based on the observation of live specimens under confined conditions. *E. cirrhosa* spontaneously spawned within the shelters arranged inside the tanks. The species do nesting and care their eggs in a similar way to *Octopus vulgaris*. During this breeding period the female remains inside the shelter, taking care of the eggs. The species easily adapted to aquarium conditions, suggesting its suitability for rearing with either experimental or commercial purposes.

Despite its importance for the fishing sector, the scarcity of studies focused on this species makes the information offered in the present work an important milestone in understanding the life cycle of *E. cirrhosa* in NW Iberian Peninsula, and, therefore, it represents a meaningful contribution to a comprehensive knowledge of the Iberian teuthofauna.

Palabras clave Cefalópodos, *Eledone cirrhosa*, ecología, ciclo reproductivo, crecimiento, edad, distribución, dieta, Península ibérica Noroeste, Atlántico Noreste.

Resumen

En la presente tesis se aborda el estudio de diversos aspectos de la ecología del pulpo blanco, *Eledone cirrhosa* (Lamark, 1798), en el noroeste de la península ibérica, desde el oeste del mar Cantábrico hasta el norte de Portugal. El pulpo blanco es un octópodo bentónico que se distribuye a lo largo de la plataforma y el talud continental en toda la cuenca mediterránea y el Atlántico noreste, desde Marruecos hasta Noruega, incluyendo las Islas Británicas e Islandia. En el noroeste de la península Ibérica esta especie es considerada como una captura incidental de la pesca de arrastre, centrada fundamentalmente en la captura de merluza (*Merluccius merluccius*), rape (*Lophius* sp.) y cigala (*Neprops norvegicus*), aunque su interés comercial varía considerablemente a lo largo de su área de distribución. Las descargas de pulpo blanco muestran un patrón cíclico, acorde con el ciclo de vida de ésta especie. De acuerdo con los informes de ICES, las descargas de *E. cirrhosa* en las subáreas VIIIc y IXa alcanzaron las 1145 t en 2013.

Tanto en lo referente a la talla máxima, como a diversos parámetros reproductivos, los ejemplares de *E. cirrhosa* de las aguas del noroeste de la Península Ibérica presentan características intermedias a las exhibidas por los individuos provenientes del norte de Europa y del Mediterráneo, sugiriendo un gradiente en las condiciones ambientales. Dicho gradiente se ve a su vez reflejado en los resultados encontrados en nuestra área de estudio.

Durante los muestreos, efectuados a lo largo de dos años, se observó además un importante sesgo en la abundancia entre sexos, siempre favorable a las hembras. La época de puesta de la especie también varía a lo largo de su área de distribución. Concretamente en el noroeste ibérico se concentra durante Mayo y Junio. De manera similar a la talla máxima, la talla de primera madurez sexual aumentó con la latitud, de manera que para las hembras fue de entre 100 y 134 mm ML y para los machos de entre 91 y 108 mm ML, dependiendo de la zona de captura.

El análisis de las capturas por unidad de esfuerzo (CPUE), obtenidas en campañas científicas efectuadas en diferentes momentos del ciclo reproductivo de la especie, muestra variaciones en la distribución y abundancia en el sector noroeste Ibérico. El modelado geoestadístico de las capturas identifica una migración estacional a aguas más someras efectuada durante la época de puesta.

Se estudió por primera vez la dieta del pulpo blanco en el Atlántico ibérico, mediante la combinación del análisis visual y molecular de los contenidos estomacales. La dieta de *E. cirrhosa* en este área está principalmente compuesta por crustáceos (74,64%), seguidos por peces (16,84%) y, en menor medida por equinodermos (5,51%), moluscos (4,92%) y poliquetos (2,09%). La dieta de la especie fue modelizada mediante Regresión Logística Multinomial (RLM). Según el modelo desarrollado, los principales factores que afectan a los patrones de alimentación fueron: el *estado de madurez*, el *caladero de origen* y la *época del año*. Los resultados obtenidos, en combinación con la bibliografía existente acerca de la distribución de las presas, sugieren que *E. cirrhosa* no es un depredador totalmente oportunista, sino más bien un generalista selectivo.

En el presente estudio se estimó por primera vez la edad de *E. cirrhosa* mediante el análisis de anillos de crecimiento en el estilete.

Un total de 122 individuos de entre 67 y 950 g fueron seleccionados de una muestra total de 2220 especímenes, mediante un análisis de progresión modal (MPA) para representar todas las clases de talla. La edad estimada varió entre 106 a 516 días y las hembras resultaron significativamente más longevas (17 meses) que los machos (14 meses). En base a las edades estimadas se calculó la fecha de eclosión, concluyendo que, aunque hay nacimientos durante todo el año, éstos se concentran mayoritariamente durante el invierno. La tasa de crecimiento relativo instantáneo, es decir, el incremento en % del peso corporal por día, estimado para clases de edad de 90 días, osciló entre 0,03% y 2,17%, disminuyendo a lo largo del desarrollo de los individuos.

Se aporta nueva información acerca del comportamiento de la especie en base a la observación en acuario de ejemplares vivos. Algunos individuos realizaron la puesta de manera espontánea en el interior de los refugios dispuestos dentro de los tanques. Se pudo comprobar que *E. cirrhosa* realiza el anidamiento y cuidado de la puesta de manera similar a *O. vulgaris*. Durante éste período la hembra permanece en el interior del refugio en el que deposita los huevos y se dedica a su cuidado. La especie tolera bien las condiciones de cautividad, lo que sugiere su aptitud para el cultivo, ya sea con fines experimentales o comerciales.

A pesar de su importancia para el sector pesquero, la escasez de estudios centrados en ésta especie hace que la información presentada en éste documento constituya un hito en el conocimiento del ciclo vital de *E. cirrhosa* en el noroeste ibérico y, por lo tanto, representa una importante contribución para el conocimiento global de la teutofauna ibérica.

Index

List of Figures

List of tables

CHAPTER 1. Foreword, objectives and structure of the thesis

Foreword

Oceans have traditionally been considered as a virtually endless source of resources; vast and productive enough to satisfy all the demands of society, which ever have had any constraint to request. Throughout the history, the role of seas in many aspects of the development of human civilization has been notable, not only as a food source, but also as a path for trade routes, channel of knowledge exchange or, in the last decades, as chief asset for the tourism sector. To our misfortune, in recent times we have witnessed how our activities have caused an increasingly clear response over marine environments. These facts have been gradually noticed by society, although unfortunately without reaching the desirable grade for promoting a reaction to an international level. The precariousness with which these changes have been perceived until now, as well as the time scale at which they occur and the intricate web of interdependence among its components, enormously hinders the study of many phenomena whose machinery only now is beginning to be posed. Variations in temperature and sea level associated to weather phenomena of unpredictable magnitude, ocean acidification, alteration in global oceans circulation or the introduction of allochthonous species; all of them have potentially destabilizing consequences over ecosystems. These factors together with the overexploitation of resources, chemical pollution and rubbish accumulation, constitute the main threats to the marine environment caused by human. Given the high number of elements involved in all these processes, complex by definition, and due to both its triggers and its ultimate consequences, eventual synergies are even more unpredictable.

In this context, our understanding of ecosystems, from a point of view of both the knowledge of its parts and how they interact, arises as an important milestone in order to pose a suitable management strategy. At the present time, technological advances constitute a crucial source of information on which modern science is based for our understanding of this complex scenario, from the development of genetic techniques, that allow the assignment of each organism to a specific group and level (i.e. species, population, breed, etc.), to Geographic Information Systems (GIS) and remote sensing, which currently allow us the continuous monitoring of many variables at different levels of resolution. Their application on the study of biological systems requires highly trained and exclusive focussed staff, a fact that has paradoxically led to a segmentation of surveys, on the other part necessary, which by themselves only can provide partial or biased answer to frequently challenging questions. Consequently, there appears to be a strong consensus around the recognition that a multidisciplinary framework for such studies is required (Coelho, 1999; Guerra, 2014).

Just over 100 million tonnes of fish are eaten world-wide each year, providing 2.9 billion people with at least 20% of their average per capita animal protein intake. This contribution is even more important in developing countries, especially small island states and in coastal regions, where frequently over 50% of people's animal protein comes from fishing (FAO, 2014). Fishing and fish farming contribute to food security by directly increasing people's food supplies, providing highly nutritious animal protein and important micronutrients while doing so. Fish food also "fill in the gaps" during times when other food is scarce. Finally, fishing and aquaculture provide jobs and income that people use to buy other foods. Concerning to cephalopods, world total catches exceeded 4 million tonnes in 2012. The share of cephalopods (squid, cuttlefish and octopus) in world fish trade was about 3 percent by value in 2012, being Spain, Italy and Japan the largest consumers and importers of these species. Catches of octopuses, which at the global level are more stable than those of squids, come mainly from the Northwest Pacific and Eastern Central Atlantic (FAO, 2014). The abovementioned might be enough to watch over the health of marine ecosystems, however apart from fishing productivity, coastal and shelf waters, combining benthic and shelf pelagic (neritic) biotas, hoard most marine biodiversity and where there is often a complex synergy of threats far greater than in offshore waters (UNEP, 2006). In fact, from a biodiversity perspective, it is not simply that coastal and shelf waters have greater species numbers and higher productivity, but also that they are biogeographically distinct from the adjacent high seas and deep benthic environments (Briggs, 1974).

Cephalopods are marine organisms, rarely present in low salinity waters, that can be found from equatorial areas to polar latitudes at all depths, from the surface layers of the ocean to deep waters (Boyle and Rodhouse, 2005). Cephalopod populations are more labile than those of long life fish species (Rodhouse, 2010) due to special features of their life cycle, such as early and/or semelparous breeding, rapid growth, short lifespan and subsequent little overlap of generations and therefore rapid population turnover. While many fish populations have been reduced as a result of overfishing, there are evidences indicating that many cephalopod stocks are increasing as a result of the reduction of predatory pressure and competition for food (Rodhouse et al., 2014). In consequence, in the last decades worldwide fisheries have increased their pressure over many cephalopod populations (Caddy and Rodhouse, 1998), by using more effective fishing gears, ships and detection methods and also by opening up new market niches for fishery products. Large-scale atmospheric and oceanic processes and local environmental variation appear to play important roles in species–environment interactions (Pierce et al., 2008). Thus, loliginid and sepiids are present in temperate and warm waters, the latter being absent in the polar areas and curiously in American waters, while oegopsid squids appear highly associated to upwelling events and water temperature seems not to be as limiting as for other groups. Distribution of octopuses is widespread, from polar to tropical areas and from shallow coastal waters to pelagic forms inhabiting open oceans at different depths. Some schooling cephalopod species, particularly squids, support important fisheries worldwide, which are largely exploited and therefore an adequate monitoring and assessment of these populations is needed. Cephalopod catches have grown progressively in the last 40 years, from about 1 million metric tonnes in 1970 to more than 4 million metric tonnes in 2012 (FAO, 2012, 2014)

The majority of cephalopods are active predators in marine environments, except nautiluses and maybe some oceanic squids (e.g. *Magnapinna* sp.). They are exclusively carnivorous animals that generally exhibit rapid growth and high metabolic rates and therefore a high energy demand (O'Dor and Webber, 1986). Cephalopods have a very important role in marine food chains, both as predators and as prey for higher predators. Most of them are largely muscular and therefore are protein-rich, and some species with large digestive glands provide a notable amount of lipids (Boyle and Rodhouse, 2005), offering a large and accessible source of energy to higher predators. Moreover, some species are the largest among the invertebrates, a fact that, in combination with its short life cycle, indicates their high metabolic rates presenting high rates of food conversion and representing a high-energy food source. Therefore, many cephalopods constitute an important source of food for many marine predators such as some seals and toothed whales and seabirds (Boyle and Rodhouse, 2005). On the other hand, the influence of cephalopods on global fisheries must be assessed not only in terms of amount of catches, but also through their influence over different fisheries. The impact of shoaling, migrating squids over other populations of fishable species can be considerable. However, the unassessed effect that these squids might produce during their early life stages in pre-recruited fish is probably much higher (Clarke, 1996).

Important cephalopod fisheries are carried out in a number of places usually linked to particular oceanographic events. Mesoscale rings and eddies linked to high-energy current systems generate local upwelling events, which support large exploitable cephalopod populations. Thus, large ommastrephid squid fisheries appear associated to western boundary current systems, such as *Todarodes pacificus* in Japan or *Illex argentinus* and *I. Illecebrosus* in eastern American coasts. Eastern boundary currents also support major cephalopod fisheries, namely *Dosidicus gigas* and *Loligo opalescens* in the western American coasts or *Octopus vulgaris* in the Eastern Atlantic (Boyle and Rodhouse, 2005). Nevertheless, although various cephalopod stocks are assessed worldwide, the current low level of fishery data collection on European cephalopods, coupled with the high data demands imposed by their short life-cycles, means that any analytical assessment is currently impractical (ICES, 2010). The assessment of cephalopod stocks in the ICES region is not only important for sustainability of the directed cephalopod fisheries (i.e. *Sepia officinalis* in the English Channel, coastal fisheries for *Octopus vulgaris* and *Loligo vulgaris*, and *Loligo forbesii* fisheries in various locations along the UK coast), but also because of the thophic role that cephalopods play in marine food webs (ICES, 2010).

However, despite their increasing demand in various sectors, cephalopods living in European waters are not generally well-managed enough, as other marine resources, as well as many teleostean fish species. The European Union marks a Common Fisheries Policy (CFP), a set of rules for managing European fishing fleets and for conserving fish stocks, aiming to ensure that fishing and aquaculture are environmentally, economically and socially sustainable and that they provide a source of healthy food for EU citizens. Because of the complexities involved, ecosystem-based fisheries management (EBFM) integrating social, economic and ecological considerations is desirable for cephalopod fisheries. An ecological approach to management is routine in many fisheries, but to be effective, good scientific understanding of the relationships between the environment, the trophic dynamics and population dynamics is essential. The present project is posed as a first step aimed towards an EBFM and therefore in the FAO Code of Conduct, which highlights the importance of managing fisheries in a responsible manner and respectful of the environment (Garcia et al., 2003). EBFM is a new direction for fishery management, essentially reversing the order of management priorities to start with the ecosystem rather than the target species (Pikitch et al., 2004). Currently, there are not yet defined the conditions needed to achieve the objective of a fisheries management focussed on a single species, regarding to the acquisition of biological parameters, tools that allow modelling and fisheries management. In particular, identification of species in the catches and biological sampling in a relevant spatial scale is something already widespread in many population units. Thus, fisheries management techniques have adapted to cephalopods populations and diagnosis of fishing pressure to which numerous species are subjected, especially, cuttlefish and octopuses. The revisions which are currently available underline that the life cycles of species may be useful for the sustainable exploitation of cephalopods as resource.

The majority of researche on cephalopods has been concerned with economically relevant and coastal-living species (Nixon, 1987), and therefore important gaps do exist on the life history of many of them. The horned octopus *Eledone cirrhosa* constitutes a good example of this fact. So, despite its relative economic interest in the Iberian Atlantic, there is an almost complete lack of information regarding relevant traits of its life cycle and ecological particularities. In Spain some papers have been published concerning to this species in the Mediterranean (Sánchez, 1986; Sánchez et al., 2004). The most remarkable contribution to the knowledge of this species in Atlantic Galician waters were *Galicia* surveys, undertaken between 1972 and 1976 by the Instituto de Investigaciones Pesqueras (Pérez-Gándaras et al., 1980). This series of surveys mainly focused on the abundance and distribution of demersal resources for fishery purposes in the region based on trawl catches. Since different surveys were carried out in different seasons, comparisons in abundance and distribution in different ontogenetic stages were possible. In Portuguese waters we can only find some scattered information in general fisheries reports from government agencies (e.g. I.N.E., 2011) and in some published works focused on modelling trawling fleet activity (Fonseca et al., 2008). In both cases *Octopus vulgaris*, *E. cirrhosa*, and most probably *Eledone moschata*, are usually reported together. As far as we know, the only work on *E. cirrhosa* in Portugal is the Master Thesis of Cabido (1984). This work, besides this species, also deals with *E. moschata* and *O. vulgaris*. Cabido's work is quite ambitious, ranging from taxonomy, morphology and internal anatomy to fishing gear, through the most relevant parameters of population dynamics (reproduction, growth, age and diet), but also aspects on the biochemical composition of the edible portion of the three species. The bibliographic effort is large and occupies much of the work. However, it contains some relevant information about *E. cirrhosa*, extracted from the analysis of the specimens caught during fishing surveys undertaken from 1981 to 1983.

This Ph. D. summarizes the main results and conclusions obtained in relation to the ecology of *E. cirrhosa*, through the analysis of its reproductive cycle, distribution and ontogenetic migration, population age structure and trophic role as a predator, as well as some behavioural observations under confined conditions. The information presented herein constitutes a milestone in our understanding of the life cycle of this species in northwest Iberian Peninsula, and it comes to fulfil an existing gap on this subject. Moreover, it represents a relevant contribution to the global knowledge of the Iberian teuthofauna.

Objectives

The objectives of this work were:

1. To define ranges of size and weight, as well as their relationships, of the horned octopus *Eledone cirrhosa* in NW Iberian Peninsula based on the analysis of samples obtained from three different locations within the studied area.
2. To characterize *E. cirrhosa* gonadic maturation process on the basis of the assessment of visual analysis of the gonads and reproductive indexes.
3. To define the spawning season and the reproductive dynamics of the species in the studied area.
4. To define spatio-temporal distributional patterns based on the analysis of geo-referenced catches per unit effort (CPUE) of experimental fishing surveys and environmental variables.
5. To estimate age and growth of the species during its juvenile and adult stages using growth increments in stylets, and to obtain new insights into population structure as

well as on several aspects of the lifecycle of this species, such as life span and hatching season, in north-western Iberian Peninsula waters.

6. To provide a comprehensive view of the role of *E. cirrhosa* in the marine trophic web through the identification of its diet composition in Atlantic Iberian waters, based on both visual and genetic identification of gut contents from wild caught animals, and testing the horned octopus feeding patterns using Multinomial Logistic Regression.
7. To provide new information on hunting, spawning and nesting behaviour of *E. cirrhosa* under laboratory conditions.

Structure of the thesis

This thesis is divided in eight chapters, including the current one, chapter one, which comprises a general foreword and justification of the work, the statement of main objectives and the structure of the thesis. The second chapter addresses the presentation of the considered area and the species under study. Each one of the following chapters deals on a particular aspect of the ecology of *E. cirrhosa* in the studied area. They all are in the format of scientific publications, four of which are already published and one submitted. Chapter eight presents the main conclusions extracted from the study.

Below is the list of chapters and topics that they address:

Chapter 1: Foreword, objectives and structure of the thesis.

Chapter 2: Introduction about the main biological and ecological traits of the species: Description, Taxonomic position, ecology and fisheries.

Chapter 3: Reproductive cycle of *E. cirrhosa* in NW Iberian waters.

Chapter 4: Distribution and ontogenetic migration events.

Chapter 5: Diet and feeding trends of *E. cirrhosa* in NW Iberian waters.

Chapter 6: Age and growth determinations and population structure of NW Iberian *E. cirrhosa*.

Chapter 7: Notes on the behaviour of *E. cirrhosa* under aquarium conditions.

Chapter 8: Final Conclusions.

References

Boyle, P., Rodhouse, P., 2005. Cephalopods: Ecology and Fisheries, Blackwell Publishing, UK.

Briggs, J.C., 1974. Marine Zoogeography. McGraw-Hill, New York.

Cabido, M.T.J., 1984. Aspectos da biologia de *Eledone cirrhosa* (Lamarck, 1798), Eledone moschata (Lamarck, 1798) e *Octopus vulgaris* (Cuvier 1797) (Mollusca : cephalopoda) na costa portuguesa, Instituto Nacional de Investigação das Pescas. Universidade de Lisboa, Lisboa, p. 94.

Caddy, J.F., Rodhouse, P.G., 1998. Cephalopod and groundfish landings: evidence for ecological change in global fisheries? Reviews in Fish Biology and Fisheries 8, 431-444.

Clarke, M.R., 1996. The role of cephalopods in the world's oceans. Philosophical Transactions of the Royal Society of London. Series B. Biological Sciences 351, 977-112.

Coelho, M.L., 1999. Trends in World (Cephalopod) Fisheries: ecosystem changes - Impacts on food supply and markets? Bulletin ACP-EU initiative 12, 31-32.

FAO, 2012. Fisheries and acquaculture statistics 2010, Rome, p. 78.

FAO, 2014. The State of World Fisheries and aquaculture, Rome, p. 223.

Fonseca, T., Campos, A., Afonso-Dias, M., Fonseca, P., Pereira, J., 2008. Trawling for cephalopods off the Portuguese coast—Fleet dynamics and landings composition. Fisheries Research 92, 180-188.

Garcia, S.M., Zerbi, A., Aliaume, C., Do Chi, T., Lasserre, G., 2003. The ecosystem approach to fisheries. Issues, terminology, principles, institutional foundations, implementation and outlook., in: Paper, F.F.T. (Ed.). FAO, Rome, Italy, p. 71.

Guerra, A. 2014. Studying problems: Interdisciplinary approaches to Cephalopod Biology. Hydrobiología, 725: 3-6.

I.N.E., 2011. Estatísticas da pesca. Instituto Nacional de Estatística. Lisboa., 104 pp.

ICES, 2010. Report of the Working Group on Cephalopod Fisheries and Life History, Sukarrieta, Spain.

Nixon, M., 1987. Cephalopod diets, Cephalopod life cycles. Academic press, pp. 201-219.

O'Dor, R.K., Webber, D.M., 1986. The constraints on cephalopods: why squid aren't fish. Canadian Journal of Zoology 64, 1591 - 1605.

Pérez-Gándaras, G., Guerra, A., Vázquez, A., Alonso-Allende, J.M., Labarta, E., Fuertes, J.R., López-Veiga, E.C., 1980. Distribución y abundancia de especies demersales de Galicia, Informes técnicos del Instituto de Investigaciones Pesqueras. CSIC, Barcelona.

Pierce, G.J., Valavanis, V.D., Guerra, A., Jereb, P., Orsi-Relini, L., Bellido, J.M., Katara, I., Piatkowski, U., Pereira, J., Balguerias, E., Sobrino, I., Lefkaditou, E., Wang, J., Santurtun, M., Boyle, P.R., Hastie, L.C., MacLeod, C.D., Smith, J.M., Viana, M., González, A.F., Zuur, A.F., 2008. A review of cephalopod-environment interactions in European Seas. Hydrobiologia 612, 49-70.

Pikitch, E.K., Santora, C., Babcock, E.A., Bakun, A., Bonfil, R., Conover, D.O., Dayton, P., Doukakis, P., Fluharty, D., Heneman, B., Houde, E.D., Link, J., Livingston, P.A., Mangel, M., McAllister, M.K., Pope, J., Sainsbury, K.J., 2004. Ecosystem-Based Fishery Management. Science 305, 346-347.

Rodhouse, P.G., 2010. Effects of environmental variability and change on cephalopod populations: an Introduction to the CIAC ’09 Symposium special issue. ICES Journal of Marine Science, 67, 1311-1313.

Rodhouse, P.G.K., Pierce, G.J., Nichols, O.C., Sauer, W.H.H., Arkhipkin, A.I., Laptikhovsky, V.V., Lipinski, M.R., Ramos, J.E., Gras, M., Kidokoro, H., Sadayasu, K., Pereira, J., Lefkaditou, E., Pita, C., Gasalla, M., Haimovici, M., Sakai, M., Downey, N., 2014. Environmental Effects on Cephalopod Population Dynamics: Implications for Management of Fisheries. Advances in Marine Biology 67, 99-233.

Sánchez, P., 1986. Distribución batimétrica y abundancia de algunos cefalópodos del mar Catalán. Investigación Pesquera 50, 237-245.

Sánchez, P., Maynou, F., Demestre, M., 2004. Modelling catch, effort and price in a juvenile *Eledone cirrhosa* fishery over a 10-year period. Fisheries Research 68, 319-327.

UNEP, 2006. Marine and Coastal Ecosystems and Human Well-being: A Synthesis Report Based on the Findings of the Millennium Ecosystem Assessment. UNEP (United Nations Environment Programme), Nairobi.

CHAPTER 2. Studied area and species introduction

The studied area

The studied area includes the continental shelf and the slope of the north-western quadrant of the Iberian Peninsula, from the western part of the Bay of Biscay to the northern third of the Portuguese coast in the NE Atlantic (Figure 2.1).

The objectives of this work fall within the ecology which is a branch of biology dealing with the relations and interactions between organisms and their environment, including other organisms. Consequently, in order to achieve a proper understanding of that holistic scenario and how its components relate, acknowlege of environmental characteristics where a specific species inhabits becomes a strong necessity. For this reason, this section will provide a descriptive overview of the study area, firstly from a geomorphological point of view, then describing its main water bodies and climatic characteristics, and finnaly a view of the main oceanographic events resulting from their interaction .

From a geomorphological point of view, the continental shelf of the studied area is considered as a flat zone and slight slope which extends from infralitoral fringe to shelf break–upper slope zone. Based on depositional processes and the morphology that originates, this zone has been classically divided in three main sectors: (i) Internal shelf, from the infralitoral border until about 50 m depth; (ii) intermediate shelf, until about 100 m depth, although high sedimentary-diversity under continental influences makes its exact limit uncertain; and (iii) external shelf, until around 200 m depth and with a merely marine sedimentation dynamics (Rey, 1993). Besides, the continental slope is the seaward border of the continental shelf, at which upper edge submarine canyons arise and extend up to 4,000 m in depth. Moreover, several bathymetric features stand out in the Atlantic Iberian Margin, beyond the shelf. The most striking is the seamounts area, with the Galicia Bank among them. The Galicia Interior Basin separates the seamounts region from the continental shelf, which is relatively narrow (30–50 km wide) in this zone, with a well-defined shelf break located at water depths of 160–180 m. The continental shelf in the western part of the Bay of Biscay attains larger extension and reaches about 70 km wide. In consequence, the continental shelf in this zone mainly comprises large submarine plains in the external shelf and upper border of the continental slope, between 100 and 500 m depth, with plain topography and mostly free of rocks. These sub-aquatic, sandy and muddy plains constitute the main fishing grounds for trawling commercial fleet (Fariña Pérez, 1996).

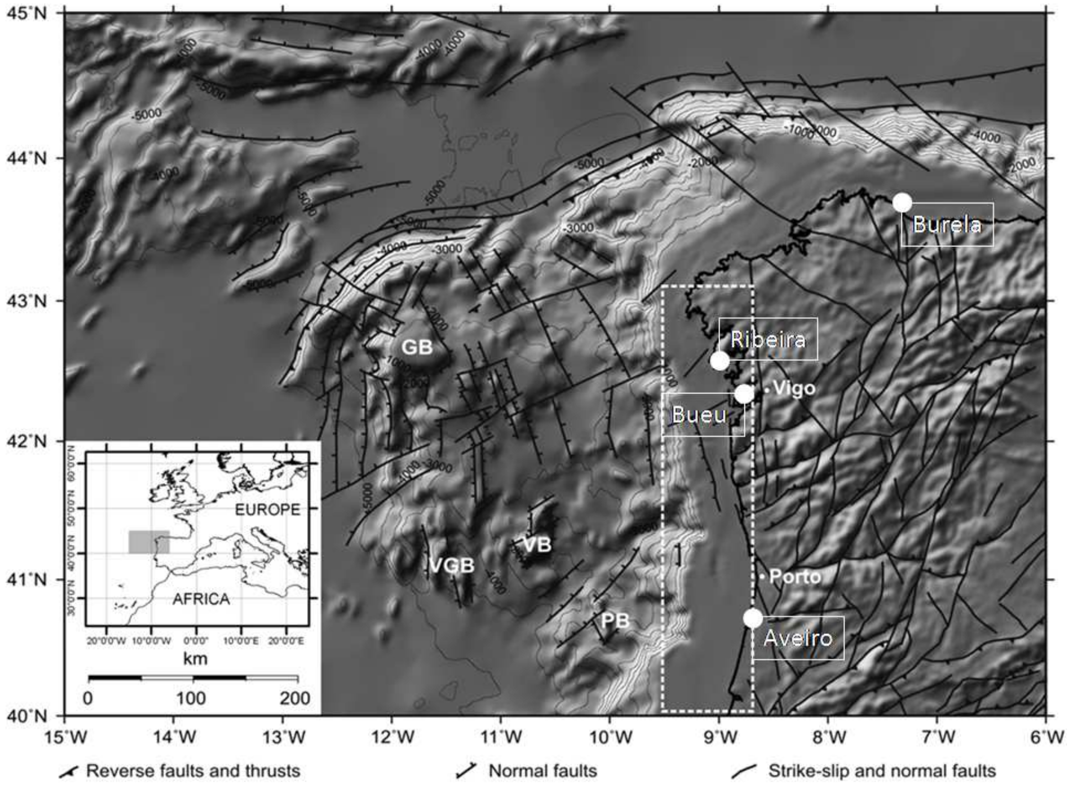


Figure 2.1. Shaded relief map from GEBCO Digital Atlas data. GB, Galicia Bank; VGB, Vasco da Gama Bank; VB, Vigo Bank; PB, Porto Bank. The fishing ports where the samples for the study were obtained are also indicated on the map. Extracted and modified from Rey et al. (2014).

Oceans are not a continuous volume of water. The ocean water is maintained in continuous motion, mainly driven by the movement of Earth's rotation (which acts differently and even in opposite way in the deep ocean and on the surface) and the constant or planetary winds. This general pattern is also affected by the location of the continents and the configuration of their coasts. Throughout this dynamic process, a number of water bodies are generated with different physico-chemical properties, which in turn define how the different water bodies behave and interact. An oceanographic water mass is an identifiable body of water with a common formation history, which has physical properties distinct from surrounding water. These properties include temperature, salinity, chemical-isotopic ratios, and other physical characteristics. Therefore, salinity and temperature define the density of a given water body and, in consequence, its relative location in the water column.

The water masses surrounding the NW corner of the Iberian Peninsula were first described by Fraga (1987), who defined their temperature, salinity and equilibrium depth. Fraga also highlighted the importance of Finisterre oceanographic discontinuity as the more important phenomenon in NW Iberian coasts. Based on this author, Rey *et al.* (2014) distinguished a number of water masses distributed in three different levels depending on the depth. Table 2.1summarizes the main characteristics of these water masses.

Table 2.1 Main water masses in NW Iberian waters (After Rey et al. 2014).

	Water massses	**Depth (m)**	**Potential temperature (°C)**	**Salinity (%)**
Surface waters	Eastern North Atlantic Central Water (subtropical branch) **(ENACWst)**	<300	13.13–18.50	35.80–36.75
	Eastern North Atlantic Central Water (subpolar branch) **(ENACWsp)**	<400	10.00–12.20	35.40–35.66
Intermediate waters	Mediterranean Water **(MW)**	400-1500	9.5–13.5	35.8–37.5
Deep waters	Labrador Sea Water (**LSW)**	1500-3000	3.4	34.89
	North Atlantic Deep Water (**NADW)**	<3000	2.2	34.91

North-west Iberian Peninsula is located on the north-eastern boundary of the North Atlantic subtropical gyre. This region has an important hydrologic and biogeochemical activity, mainly attributable to the coastal upwelling system which seasonally takes place in the area, generating an important primary production, and related to the presence of Eastern North Atlantic Central Water (ENACW) near the coast. This Canary-Iberian upwelling system (Figure 2.2) comprises NW African coasts and SW Europe. Latitudinal change that occurs along this stretch of trade winds causes the seasonal upwelling at either ends of the subtropical gyre, while in the central part the upwelling is relatively constant throughout the year (Wooster et al., 1976). Northern winds blowing parallel to the coast drag warmer, usually nutrient-depleted superficial water layers westwards, due to Coriolis effect, to being replaced by dense, cooler, and nutrient-rich ENACW. This coastal upwelling occurs mainly during spring–summer months along the western and northern coast of the Iberian Peninsula, although it is more frequent and intense along the western coast than in the northern one (Alvarez et al., 2010). Thus, our study area represents a gradient with respect to the influence of the coastal upwelling. Moreover, coastal upwelling follows the north–south migration of the atmospheric pressure systems along the eastern boundary of the North Atlantic sub-tropical gyre (Arístegui et al., 2009). North

Atlantic oscillation (NAO) modulates climate and oceanography variability of the region, particularly in winter, and is defined by the relative difference of atmospheric pressure at sea level between the Icelandic low and the Azores high pressures. Based on fluctuations in both intensity and relative position of these pressure systems, the strength and direction of westerly winds and storm tracks across the North Atlantic, two phases can be identified: one positive and other negative. During the positive phase, winters are cold and dry with weaker winter storms. During the negative phase, winters in NW Iberia are wet and warm with intense storms increasing riverine discharge and sediment resuspension on the shelf (Otero et al., 2010; Trigo et al., 2004).

Furthermore, a number of other oceanographic events have effects in the NW Iberian corner, especially in the continental shelf dynamics. The Atlantic Iberian sector may be separated into the Galician and Portuguese (west coast) sub-regions, both of which are strongly influenced by freshwater runoff causing so-called river plumes. Although these sub-regions are quite similar in most respects, the former is distinguishable by the presence of the Galician Rías, wich are drowned tectonic valleys of moderate depth with a positive estuarine pattern influenced by the fresh water inputs due to rivers contribution (e.g. Verdugo, Oitavén, Tambre, Lérez, Ulla, etc.). This estuarine circulation strongly interacts with the shelf up/downwelling circulation and biogeochemistry (Arístegui et al., 2009). The mark between these two sub-areas is the Miño River with an annual average discharge of 430.8 $m^3 s^{-1}$ (ranging 100-1,000 $m^3 s^{-1}$). In the Portuguese subarea the main river is the Duero with a flow of 710 $m^3 s^{-1}$ (ranging 50-3,000 $m^3 s^{-1}$). Fresh water discharge of both rivers is hightly dependent of the rainfall patterns in their respective hidrographic basins (Rey et al., 2014).

Together with seasonal upwelling and river plumes, the Iberian Poleward Current (IPC) is the last major coastal process occurring in the area. IPC appears like a warm anomaly flowing poleward mainly in December-January, although it actually starts as an undercurrent with a core at around 200 m depth during late summer. By this time, the shelf and upper slope are still occupied by the southward flowing upwelling jet. Starting from December, average winds change from northerly to southerly direction promoting poleward flow over the shelf and upper slope. During these months, under favorable wind conditions, the IPC core migrates vertically, from the shelf break depths to the surface, becoming a surface intensified jet. From February to May, the current weakens and part of it propagates offshore (Teles-Machado et al., 2015). So that, IPC is a narrow, slope-trapped tongue-like structure that flows northerly along a distance in excess of 1,500 km off the coasts of the Iberian Peninsula and SE France. It is a salty surface current (about 200 m deep), geostrophically trapped by the bathymetric discontinuity at the shelf break–upper slope zone and capable of transporting very fine-grained detrital material in suspension (Rey et al., 2014).

Specimens of *E. cirrhosa* for this study were obtained at three-four ports (Burela, Ribeira or Bueu and Aveiro, Figure 2.1) distributed along the western part of the Bay of Biscay and the northern third of the Portuguese coast. Given the location of sampling ports and considering depths between 50 and 1,000 m, the studied area comprises a surface of aproximately 2,300 km^2 with about 2,500 km of coastline. The above mentioned oceanographic features makes this zone a specially suitable area for studying marine organisms, due to its high productivity, and also its interaction with abiotic features along this environmental gradient.

Bearing in mind this scenario, I aimed to study the ecology of the horned octopus (*Eledone cirrhosa*, Lamarck, 1798), which inhabits the continental shelf and slope of the northwestern Iberian Peninsula, from the western part of the Bay of Biscay to the northern Portuguese coast.

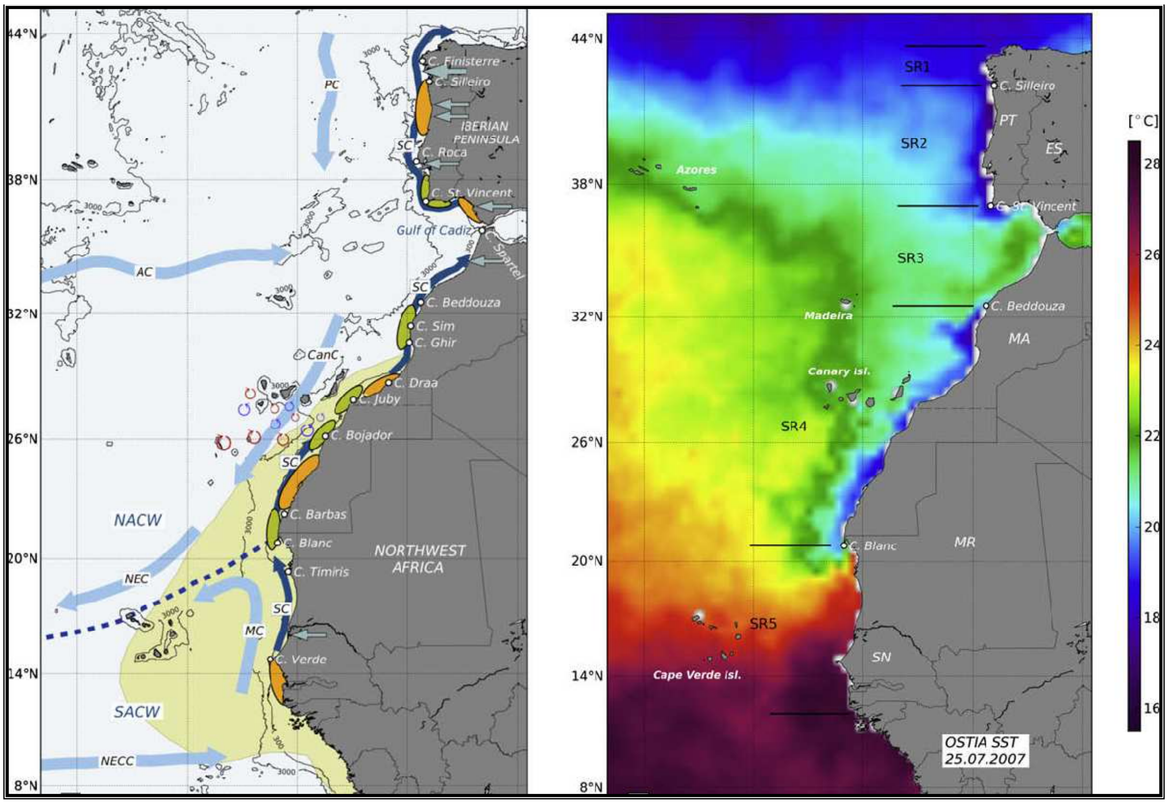


Figure 2.2. (A) Schematic map of the Canary Basin showing the main currents (light blue: surface currents; dark blue: slope current), major capes, freshwater (blue arrows) and dust inputs (>10 g m_2 y_1 shaded yellow), retention (orange) and dispersion (green) zones on the shelf, frontal zone between water masses (dashed blue lines) and mesoscale eddies (blue: cyclones; red: anticyclones) south of the Canary Islands. NACW: North Atlantic Central Water; SACW: South Atlantic Central Water; AC: Azores Current; CanC: Canary Current; MC: Mauritanian Current; NEC: North equatorial Current; NECC: North equatorial Countercurrent; PC: Portuguese Current; SC: Slope Current. **(B)** Map of sea-surface temperature over the study area on 25 July 2007 from OSTIA (Stark et al., 2007), showing the five sub-regions described for this area (SR1: Galician; SR2: Portuguese; SR3: Gulf of Cadiz; SR4: Moroccan; SR5: Mauritanian). PT: Portugal; ES: Spain; MA: Morocco; MR: Mauritania; SN: Senegal. Extracted from Arístegui et al. (2009).

The species

The horned octopus *Eledone cirrhosa* is also known in England as lesser octopus or curled octopus; polvo-do-alto or polvo cabeçudo in Portugal; pulpo blanco in Spain and cabezudo in Galicia (NW Spain); élédone commune, poulpe blanc or pieuvre blanche in France; moscardino bianco in Italy; and μοσκιός [moschios] in Greece.

The first complete description of this species was done by Isgrove in 1909. That document addressed an external description of the animal, as well as a comprehensive review of their internal organs and anatomical organization. Since then, a series of manuscripts have been published focussing on this species in particular or targetting a wider taxonomic scope (e.g. Naef, 1923; Robson, 1929, 1932), becoming key guides for teuthologists.

Description

Eledone cirrhosa is a medium-sized octopod, with a maximum body weight <1 kg in the Mediterranean and up to 2 kg in the North-Eastern Atlantic (Belcari et al., 2015). As it occurs whit all cephalopods, *E. cirrhosa* is a dioecious species (separate sexes). The especies shows a slight sexual dimorphism concerning individual size, with females larger than males. The mantle, that constitutes the visceral cavity, has a finely grained external appearance and it is broad and ovoid, with a peripheral skin ridge separating the dorsum and the belly. Coloration presents a countershaded pattern with a reddish-brown dorsal color with scattered small white markings. The ventral area is white, with a greenish iridescence. The head is narrower than the mantle and two big well developed eyes with respective retractile supraocular papilla are located on top of the dorsal side. The eight circumoral appendages have a single row of suckers and emerge from the head. They are of intermediate size, comprising aproximately 60% of the total lengh, and subequal. The third right arm in males is hectocotilyzed, that is modified for copulation, smaller than the corresponding opposite (69–76%) and with a very short ligule (Figure 2.3.I.C). The distal suckers of not modified arms form a single row of papillae (Fig 2.3.I.B). The skinny membrane that connects arms each other (umbrella) reaches 21-41% of the length of the arms.

Eledone cirrhosa is able, as well as many other cephalopods, to modify the texture of the skin, take a number of different body patterns, and change its colour through the action of pigmented epithelial structures arranged in several layers. The combined action of these mechanisms is under nervous control and through them the animal is able to express a number of characteristic behaviours (see Hanlon and Messenger, 1996).

The preserved specimens may be difficult to differentiate from *E. moschata*, deemed a twin-Eastern Mediterranean species of the Atlantic *E. cirrhosa* (Bello, 2003), although differences in coloration and behavior does not pose a likelihood of confusion in fresh specimens (Boyle, 1997).

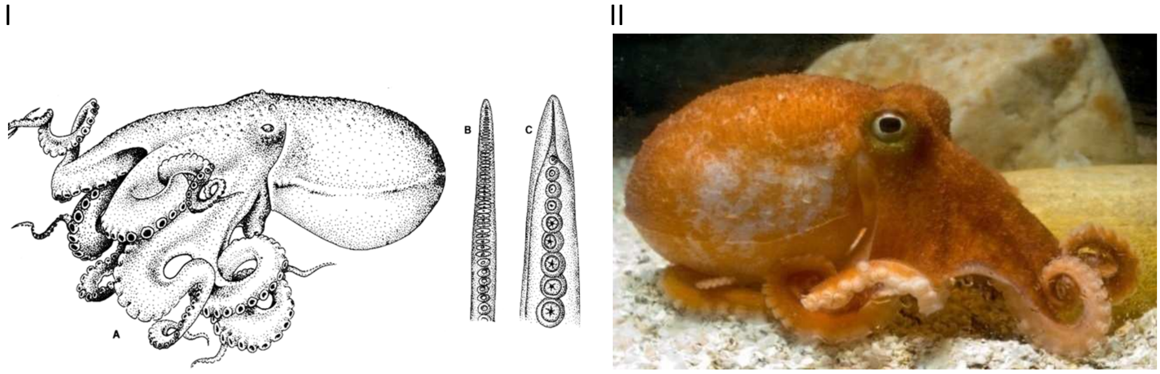


Figure 2.3. (I) *Eledone cirrhosa* (Lamarck, 1798) lateral view (A). Tip of an adult arm (B) and hectocotylus (C). (After Guerra, 1992). **(II)** Specimen of Eledone cirrhosa photographed in aquarium.

The internal organs are placed inside the mantle cavity. The digestive and reproductive systems occupy most of such cavity. The digestive system starts in the mouth, located in a muscular buccal mass, which houses two chitinous jaws, similar to parrots' beak, and a radula. The beaks are one of the few hard structures of cephalopods and its morphology has been described for use in systematic diagnosis (e.g. Clarke, 1986; Pérez-Gándaras, 1983) as well as for estimating individual age by counting the number of growth increments. The radula is also important in taxonomical studies. Three paired salivary glands are located around the buccal mass. The function of these salivary glands is the excretion of mucus, digestive enzymes and neurotoxins. The oesophagus connects the mouth to the crop, across the brain and the hard cartilaginous skull. The crop leads to a muscular stomach that in turn connects with a gastric spirally-shaped caecum. The digestive gland connects the digestive tract between the stomach and the caecum through two digestive ducts. The ink sac and its duct are located underneath the digestive gland. The presence/absence of an ink sac has been considered a major taxonomic character in Octopodidae. On the other hand, ink clouds provide a useful tool for evading potential predators. The last section of the digestive duct is the intestine that ends at the anus, which is located next to the funnel in the ventral side of the animal.

The *E. cirrhosa* reproductive system also occupies a large space within mantle cavity. The females' reproductive system is composed by a single ovary and a pair of oviducts, each with an oviducal gland (Figure 2.4.A). The oviducal glands function is to form the covering layers around the eggs. The males' reproductive system comprises a single testis and the Needham's complex (Figure 2.4.B). According to Boyle and Knobloch (1984a), sperm is shed into the envelope of the testis from which it enters the opening of the proximal vas deferens. This is a delicate and highly folded tube which is seen as a tight knot of convoluted sperm rope in mature animals. The vas deferens then becomes firmer and enlarges into a glandular area which we refer to as the spermatophoric gland system I. A distal vas deferens leads to a second, more complex, spermatophoric gland system II which opens into the curved spermatophoric sac, where already formed spermatophores are stored.

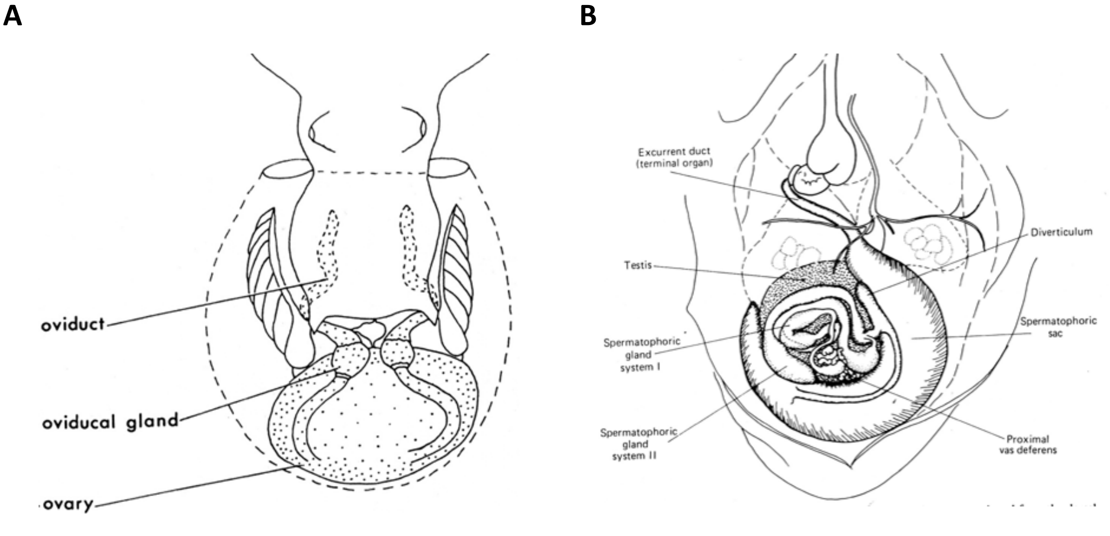


Figure 2.4. Schematic view of the reproductive tract of a female **(A)** and a male **(B)** of *Eledone cirrhosa*. (After Boyle and Knobloch, 1982b, 1984a)

Mating process in this species has been described as *mounting* (Boyle, 1983b; Orelli, 1962), i.e. the male approach the female from behind, enveloping her with his arms and web and inserting the hectocotylus into the right side opening of the mantle cavity (Orelli, 1962). The spermatophores of the males are transferred from the terminal organ of the excurrent duct to a groove along the hectocotilized arm formed from a fold of skin. The spermatophores are pumped along the arm by muscular contractions, to be released by the tip of the hectocotilus into the mantle of the female and pass completely along the oviduct and through the oviducal glands before finally bursting in the ovary itself, where the oocytes are stored. This makes the difference with other octopods, since *Eledone* is the only genus in which this transference is directly made, while in the remaining octopod groups, spermatophores are stored in the oviducal glands until fertilization. In consequence, the oocytes in this species are fertilized before beginning their passage down the oviduct (Boyle and Rodhouse, 2005).Thus, during reproductive sampling for the present study, a number of mature ovaries were found with several spermatangia (everted sperm sacs) inside (Figure 2.5).

Figure 2.5. Image of a dissected ovary and spermatangia found inside it.

As the majority of cephalopods, octopods have three hearts, one main two-chambered heart (the systemic heart) and two smaller branchial hearts, one next to each set of gills.

The number of lamellae per hemibranch is a taxonomic character, which in *E. cirrhosa* ranges between 11 and 13. The closed circulatory circuit sends oxygenated blood from the gills to the atrium of the systemic heart, then to its ventricle which pumps this blood to the rest of the body. Deoxygenated blood from the body goes to the branchial hearts which pumps the blood across the gills to oxygenate it, and then the blood flows back to the systemic atrium for the process to begin again (Wells, 1980). Three aortae leave the systemic heart, two minor ones (the abdominal aorta and the gonadal aorta) and one major one, the dorsal aorta which services most of the body (Smith, 1981). The blood of the cephalopods is composed of copper-rich hemocyanin which is less efficient than the iron-rich hemoglobin of vertebrates and thus does not increase oxygen affinity to the same degree. Thus, cephalopod blood can transport less than 5 millilitres of oxygen per 100 ml of blood, compared with about 15 vol% in fish.

The excretory system is built around an appendage of each branchial heart. These long, ciliated ducts filter the blood into a pair of kidney sacs while actively reabsorbing glucose and amino acids into the bloodstream. The renal sacs actively adjust the ionic concentrations of the urine, and actively add nitrogenous compounds and other metabolic waste products to the urine. Once filtration and reabsorption are complete, the urine is emptied into the octopus' mantle cavity via a pair of renal papillae, one from each renal sac (Wells, 1978).

As molluscs, the cephalopods have a shell, which can be more or less reduced depending on the systematic group. In octopods, and particularly in *Eledone* species, the shell is embryologically derived from the invagination of a reduced ectodermic area (Sacarrao, 1951) and it is reduced to two cartilaginous sticks called stylets, embedded in the mantle musculature. These structures grow, as well as the beak, by successive deposition of increments, which has been proved to be produced at regular time intervals and make them suitable as individual chronological markers. Even though the use of stylets for ageing cephalopods is relatively recent (the first validation corresponds to Doubleday et al., 2006), the studies of Cabido (1984) suggested their feasibility to be used for this purpose. This author also studied stylets biometrics, showing some sexual dimorphism. However, although growth rings were found in the stylets, the age of the specimens could not be estimated since samples showed rapid degradation due to the lack of an appropriated technique. Unfortunately, to date, this work has not been considered as relevant to the scientific community. Several years later Moriyasu and Benhalima (1993) described the morphology of *E. cirrhosa* stylets and noted their usefulness for estimating growth. Nowadays, stylet growth rings have been succesfully used and validated for age determination in three octopod species, namely *Octopus pallidus*, *O.vulgaris* and *O. maya* (Doubleday et al., 2006; Hermosilla et al., 2010; Rodríguez-Domínguez et al., 2013).

The neuro-sensitive system of cephalopods is the most developed among the invertebrates, providing them with a large cognitive potential and causing high complexity behavioural patterns. The central nervous system of *E. cirrhosa* is rather similar than that of *O. vulgaris* (Nixon and Young, 2003). A number of gangliar masses, comparable to cerebral, medial, pleural and visceral ganglia, can be identified in the neural organization of the species. Nevertheless, they have lost their integrity as individual nodes, and have subdivided and reorganized in a circumoral nerve center that works like a complex brain.

Among cephalopod sensorial systems, vision is perhaps the most remarkable and indeed cephalopods' eyes are the most complex among invertebrates. Most species are visual-dependent hunters and their brain has large visual areas (Jozet-Alves et al., 2014). Cephalopods have a camera-type eye which consists of an iris, a circular lens, vitreous cavity (eye gel), pigment cells, and photoreceptor cells that transfer light from the light-sensitive retina into nerve signals, which travel along the optic nerve to the brain. Therefore, eye structure of cephalopods is very similar to those of vertebrates, in fact visual acuity in *Octopus* is comparable to that of fishes and aquatic mammals (Muntz, 1991). The eye of *E. cirrhosa* has an elipsoidal lens with a contractile, horizontal, slit-like diafragm (Nixon and Young, 2003), making it an effective organ in reducing optimal distortion. Althoug the rought grain of the retinal mosaic suggest that its potential to generate high-resolution images is limited (Nixon and Young, 2003), is far less than would be needed to limit acuity to this level (Sroczynski and Muntz, 1985). There is no evidence that cephalopod see colours but they can perceive light polarization (Hanlon and Messenger, 1996), which can be considered as analogous of color and is arguably involved in breaking camuflage of potential prey, intraspecific communication or orientation behaviour (Jozet-Alves et al., 2014). In addition to the normal retinal photoreceptors of the eyes, most cephalopods, *E. cirrhosa* among them, have small groups of extraocular photoreceptors or photosensitive vesicles, which in adult stages of benthic and pelagic octopods consist of a single pair of organs located inside the mantle cavity, more specifically on the ventral posterior margin of the stellate ganglion (Cobb et al., 1995b).

Detection of chemical cues can be either through contact chemorecepcion (gustation), through chemoreceptors located on the suckers and lips (Boyle, 1986a), or distance chemoreception (olfaction) by means of olfactory organs close to the eyes (Jozet-Alves et al., 2014). *Eledone* is sensitive to chemicals such as proline, glycine and especially betaine, all of which are constituents of arthropod flesh (Boyle, 1986a) and experiments on *Octopus* ventilation rates showed that animals increased ventilation rate when exposed to waters in which crabs have lived (Boyle, 1983a).

Eledone cirrhosa behaviour has been mainly studied by observing specimens kept in aquarium (e.g. Boyle and Dubas, 1981; Boyle and Knobloch, 1981) and often confirmed by anatomical observations (see Nixon and Young, 2003). Reproductive behaviours include agonistic (i.e., the complex of behaviours including fighting, threat, appeasement and avoidance, usually among males), courtship (i.e. all behaviours that precede and accompany the sexual act), mating (i.e. copulation, which in cephalopods does not usually include fertilization), and any form of parental care if it really occurs (Hanlon and Messenger, 1996). This species has been described as a *boring* species, maybe due to its nocturnal pattern of activity (Cobb et al., 1995a; Cobb et al., 1995b). Nevertheless, the number of chromatic components and body patterns in *E. cirrhosa* compared with other octopoda is intermediate, with 15 chromatic components and 2 body patterns (Hanlon and Messenger, 1996). Even based on the structure of the visual and chemotactile systems *E. cirrhosa* has been suggested to be capable of learning, like *O. vulgaris* (Nixon and Young, 2003).

Systematics

Systematic characters may be used for taxonomy or for classification and phylogenetic analysis. An overiew of cephalopod characters was given by Nixon (1998). This author emphasized three things: i) the relatively small number of hard structures in this soft-body molluscs; ii) the difficulties to found good characters in the radula, gladius, cuttlebone, eye lenses, mantle-and nuchal-locking apparatus, beaks, dermal structures, cephalic cartilage, hooks, statoliths and stylets, which are the hard tissues found in cephalopods, except the external shell of nautiluses and the calcified shell of *Spirula* and *Argonauta*, and iii) that an ideal description of a cephalopod will include morphological, meristic, ecological, ethological, and biochemical characters. Since hard-or tough-tissue structures are better to withstand adverse conditions, such as those experienced during capture, and they survive largely without distorsion or loss material, even after inmersion in the digestive juices of a predator, postmortem changes, and even fossilization, they were used during the last three or four decades for systematic, taxonomic and phylogenetic studies. However, development and availability of other techiques, such as DNA sequencing and underwater filmation are changing the perspective and accuracy of this kind of studies.

Moreover, scientific sampling gears have limitations for capturing certain types of species (mainly fast-swimming ones) and many of these captured specimens usually are seriously damaged, leading to loss certain taxonomic characters. On the other hand, throughout history, the finding of early life stage forms usually has caused confusion in the scientists, and genera and species have been sometimes created on the basis of different life stages (Boyle and Rodhouse, 2005).

All extant cephalopods (including octopuses, squid, cuttlefish and sepiolids) except *Nautilus* species are classified in a single subclass: Coleoidea. Because of the very large number of morphological differences between the many coleoid lineages, the lack of shared characters, the uncertainty regarding character homology and the differing rates of evolution across groups, biologists have struggled to understand many of the evolutionary relationships among taxa (Allcock et al., 2015).

The scarcity of meaningful morphological characters is especially sharpy in some groups within the order Octopoda, for instance in Eledoninae. This fact leads the traditional taxonomic classification system to particular problems in this group. Thus, characters as the presence or absence of an ink sac and the number of sucker rows on each arm have traditionally played major roles in attempts to classify groups within the family Octopodidae, in which *E. cirrhosa* is included. As above exposed, the recent introduction of molecular techniques have called into question all previously stated about this issue and, at the same time, have provided us a series of more precise tools for analyzing phylogenetic status of organisms.

Thus, although the taxonomy and classification of living cephalopods are still unfixed (Allcock et al., 2011) and this particular group is undergoing considerable revision, mainly by genetic techniques (Lindgren et al., 2012; Strugnell et al., 2014), the currently widely accepted classification is as follows:

Phylum ***MOLLUSCA***
 Class: ***CEPHALOPODA***
  Subclass: ***COLEOIDEA***
   Order: ***OCTOPODA***
    Superfamily: ***OCTOPODOIDEA***
     Family: ***ELEDONIDAE***
      Genus: ***ELEDONE***

Ecology

Demographic ecology: Reproduction, Growth and Population dynamics.

Cephalopods are gonochoric animals (sexes are separated), and there are no hermaphroditism or sex-change. The horned octopus, as well as most cephalopods, is a semelparous or spawning once species; that is, it has only one reproductive event, shortly after which adults die. *E. cirrhosa* can be clasiffied as an “intermittent terminal spawning” *sensu* Rocha et al., (2001), with group synchronous ovulation, monocyclic spawning and no growth between egg batches. In consequence, the only reproductive event acquires a remarkable importance and has traditionally been a major theme in the studies on cephalopods life history. Reproductive traits of this species have been studied through experiments with captive specimens from Mediterranean sea (Mangold et al., 1971) and the Atlantic ocean (Boyle and Knobloch, 1983, 1984a, b). In *E. cirrhosa,* as well as in most cephalopods, sexual maturity can occur at a wide range of body sizes, and males reach sexual maturity earlier than females and at smaller sizes.

Gonadal maturation process implies a gonad enlargement and therefore maturity state has been traditionally assessed by the "Gonadosomatic Index" (Weight of the gonad expressed as percentage of body weigth). In Octopodidae the "Ovary Index" in mature specimens invariably ranges between 10-40% (Mangold, 1987). Hayashi index (Hayashi,

1971) developed for *Todarodes pacificus* and employed for the first time in an octopod by Guerra (1975), has been also used to assessing maturity. This index relates testis and the Needham's complex weights in males, or ovary and oviducts (including oviductal glands) weights in females. During the 60s and 70s, Martin Wells conducted a series of experiments on hormonal regulation of the maturation process in octopuses, whose results were compiled in a single volume (Wells, 1978). Afterwards, Boyle and Thorpe (1984) found an association between optic-gland enlargement and gonadal development in female octopuses. However, Boyle and Knobloch (1984b) noted a number of other possible causes for early maturity on aquarium-held animals, such as confinement conditions through feeding, lighting conditions or nitrogen levels variation in relation with the wild environment, as a trigger of maturation process or inhibitor of egg-laying. Just as environmental conditions in captivity affect the process of maturation, they also modulate population dynamics in the wild, influencing the duration of spawning and brooding seasons and therefore changing the age structure of populations (Belcari et al., 2002). Thus, Mediterranean *E. cirrhosa* reach sexual maturity earlier (spring–summer) in western waters (Sanchez, 1976; Belcari and Sartor, 1999) than further east (summer–autumn) (Lefkaditou et al., 2000). In the North Sea, although mature individuals are present all year around, a peak is observed from July to September, with spawning occurring shortly thereafter (Boyle, 1983b; Boyle and Knobloch, 1983). In Portuguese waters maturity peak occurs in May-June although it extends from March to November (Cabido, 1984). This variability in the timing of maturation suggests that its regulation is complex and depends to some extent on external factors, which in turn varies along the range of the species.

Fecundity is given by the number of offspring that a living being can beget. Since direct observation of this variable is often not feasible in *E. cirrhosa*, as well as in many others, and for comparative purposes, two terms has been coined in which the concept of fecundity is based: Potential Fecundity (PF) and Relative Fecundity (RF). PF refers to the total number of mature oocytes from the ovary of a mature female and RF expresses the PF in relation to the body weight of the animal (nº of oocites/g). However, fecundity may be underestimated because spawning may have already started (Mangold, 1987) or overestimated due to the reabsorption of oocytes through atresia (Boyle and Chevis, 1992). Therefore, *E. cirrhosa* fecundity data reported in the literature widely vary, not only by geographic location but also between studies performed in the same locality. Thus, Boyle et al. (1988) noted that potential fecundity was close to 9000 eggs/female in Aberdeen and 5500 oocytes/females in Banyuls, while some years before estimates ranged from 2200 to 55000 oocytes /female in Aberdeen (Boyle and Knobloch, 1983).

As observed by Boyle (1981), specimens maintained under rearing conditions can lay their eggs on the tank walls. This observation suggests that *E. cirrhosa* lays its eggs over rocky substrate, and centainly not in muddy or sandy bottoms. Eggs are laid in strings, and each string contains between 25 and 30 eggs (Boyle, 1997). Fertilised eggs hatch after 3–4 months at 14–18°C (Mangold et al., 1971).

In octopods the number and size of eggs varies widely. Thus, octopods produce from 10 to 500 000 eggs (Nixon and Young, 2003), with a size ranging from less than 1mm to over 40mm in length, resulting in either a planktonic or a benthic offspring (Hochberg et al., 1992; Mangold, 1987). Egg length in *E. cirrhosa* (7.5 mm) is considered intermediate in size between small-egged (e.g. *Octopus vulgaris*; 2 mm) and large-egged (e.g. *Eledone moschata*; 13-15 mm) cephalopods. This fact, together with a low egg index (relationship between egg size and adult mantle length), indicates that newly hatchlings are pelagic, although this phase probably would last only few days during which most of them remain relatively close to the bottom (Boyle, 1983b).

We mean “larvae” as the juvenile stages of animals with indirect development (metamorphosis) and having an anatomy, physiology and ecology different than adults. Since there is not a metamorphosis in cephalopods, they lack a true larval stage. Nevertheless, some species have planktonic live stages, while in others octopods (e. g. *E. moschata*) the newly hatchings are very similar to the adults and live in the same habitats. Therefore, the term “paralarvae” was coined to define those young cephalopods in the planktonic stages between hatchling and subadult (Young and Harman, 1988). Paralarval forms have been observed only in members of the orders Octopoda and Teuthida. Since their lifestyle often differs greatly from that of adults and that its size is considerably smaller, obtaining hatchlings usually involves great difficulties and their ecological traits are a subject of study by itself. Therefore, very little is known about the life history of early life stages of *E. cirrhosa*. The following table (Table 2.2) sumarizes the scarce existing records.

As previously mentioned, planktonic forms of *E. cirrhosa* last a relative short period of time and most probably live near the sea floor. A description of these newly hatchings can be found in Hochberg et al. (1992) (Figure 2.6). Several features diferentiate paralarval and adult anatomy, derived from its different lifestyle. An example is the number of row suckers in some *Octopus* species, which are typically present in a single straight row and being increasing in number and gradually becoming apparent the subsequent two rows. Neverthesless, unlike other species, *E. cirrhosa* retain the single row of suckers into adulthood (Villanueva and Norman, 2008) and this characteristic constitutes an identificative taxonomic character.

Table 2.2 Sampling methods, number of early-life stage individuals collected and abundances of *Eledone cirrhosa* from the literature. Modified from Villanueva & Norman (2008).

Gear	Horizontal or vertical	Depth range (m)	Day/ night	Number of individual collected	Abundances	Observations	Geographic area	Reference
Np.	Np.	0-505	Np.	15	Concentrated between 0-100 m	All year round	NE Atlantic	Russell (1921) quoted by Boyle (1983)
Planckton net	Np.	0-200	Np.	118	Midwater or near the bottom, rarely at surface	Higher from May to August	NE Atlantic	Stephen (1944)
Variety of plancton nets	Horizontal and oblique towns	0-200	Np.	62	Largest individuals near to the seafloor	Higher from May to June	NE Atlantic	Collins *et al.* (2002)

The paralarval internal anathomy generally follows similar organization than that of the adult. The paralarvae nervous system matches the general pattern described for adults (Wells, 1978), but it is comparatively larger by volume. Thus, the relative proportions of the lobes of the paralarval brain are markedly different from those of juveniles or adults. In *O. vulgaris* and *E. cirrhosa* these differences have been related to morphological development and changes in mode of life (Villanueva and Norman, 2008). Indeed, the palliovisceral lobe and the higher motor centres of the *E. cirrhosa* hatchling form a relatively larger portion of the brain than in the adult. This is likely to be related to the locomotor activity during the planktonic phase of this species (Nixon and Young, 2003). In *O. vulgaris* buccal and basal lobes are larger at hatching than in juveniles, while the brachial lobes increase their volumen from 8% of the total volume of the brain to 13% at settlement, coinciding with the rapid growth of the arms and suckers and the development of the tactile sense that is characteristic of the octopus's benthic life, reaching 18% in the adult (Nixon and Mangold, 1996). Paralarval and juvenile phases commonly exhibit a rapid, exponential growth, slowing down afterwards during breeding (Jackson, 2004).

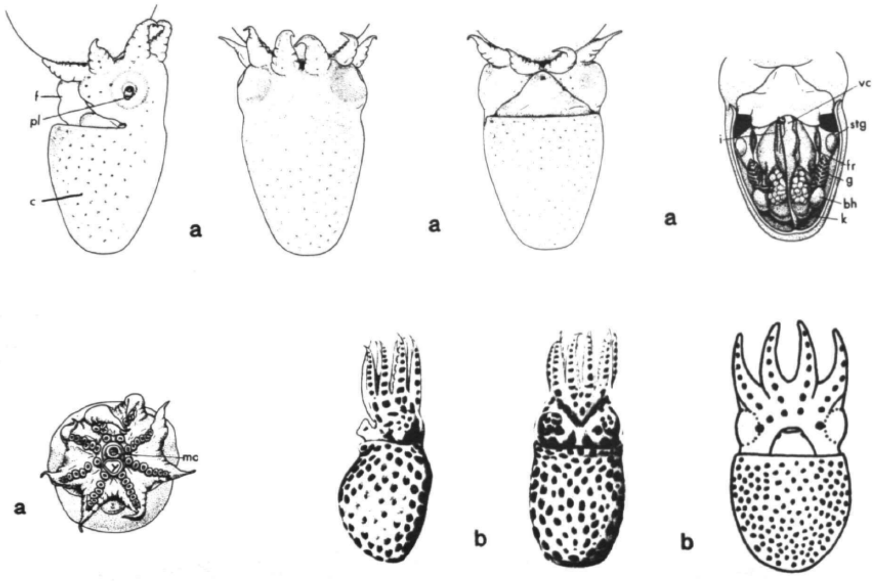


Figure 2.6. *Eledone cirrhosa* a, lateral, dorsal, ventral, mantle cavity and oral views, prehatchling (Stage XIX), 3.2 mm mantle length (ML), Mediterranean (from Mangold et al., 1971) (bh = branchial heart; c = chromatophore; f = funnel; fr = funnel retractor, g = gill; i = intestine; k = renal sac; mo = mouth; pi = primary lid; stg = stellate ganglion; vc = vena cava; y = yolk); b, lateral, dorsal, and ventral views, hatchling, 3.8 mm ML, English Channel (from Rees, 1956; ventral view, Hochberg original). (After Hochberg et al., 1992).

E. cirrhosa growth has been studied in several works in different parts of their geographic range. Boleda (1976) studied the growth of sub-adults and adults in the Iberian Mediterranean. She indicated the simultaneous appearance of two kinds of sizes and estimated a growth rate of about 1 cm per month in individuals of up to one year, slowing down for bigger individuals. Leftkaditou & Papaconstantinou (1995) in the Eastern Mediterranean, found growth rates of 10-20 mm each three months in small individuals. These studies seem to indicate slight diferences in growth rates between western and eastern individuals. Nevertheless, growth rate estimation on the basis of mantle lenght measures is unadvisable nowadays, since it may causes biases when performed over soft-bodied animals and weight is a much more sensitive and dynamic measure of growth (Forsythe and Van Heukelem, 1987). Boyle in Scotland estimated growth rates based on Body weight (BW) calculated at weekly intervals. Specimens showed growths between 0.33 and 1.06 % BW x day^{-1}.

Cephalopods have a short lifespan, little overlap of generations, and high vulnerability to predation and/or variations in environmental conditions. In contrast, they exhibit great physiological plasticity (O'Dor and Shadwick 1989), which results in substantial individual variability in growth rates and maturity. This characteristics result in a wide inter-annual fluctuation in abundance (Boyle and Boletzky, 1996). Fecundity is clearly linked to the survival strategy of the species, and it is dependent on their size and mass which, in turn, are often temperature-related (Lipinski, 1998). On the other hand, the existence of a planktonic paralarval stage, which spreads in a relatively pasive way, makes discrete populations typically difficult to identify, and a metapopulation concept may, therefore, be useful (Lipinski, 1998).

A life-span of between 18 and 24 months with individuals dying shortly after spawning was proposed for *E. cirrhosa* (Boyle, 1983b). Although, as previously mentioned, the reproductive cycle for this species in the northern Atlantic extends all year round, the proposed cycle was based on the assumption of sexual maturity occurring during summer and that spawning is likely shortly afterwards. Hatchlings would appear in the water column 3 or 4 months later and then, after a short planktonic phase apparently inhabiting waters close to the sea floor, they settle. According to this approach, recruitment would occur in March-June of the following year. A schematic view of the proposed life cycle of the species is shown in the Figure 2.7.

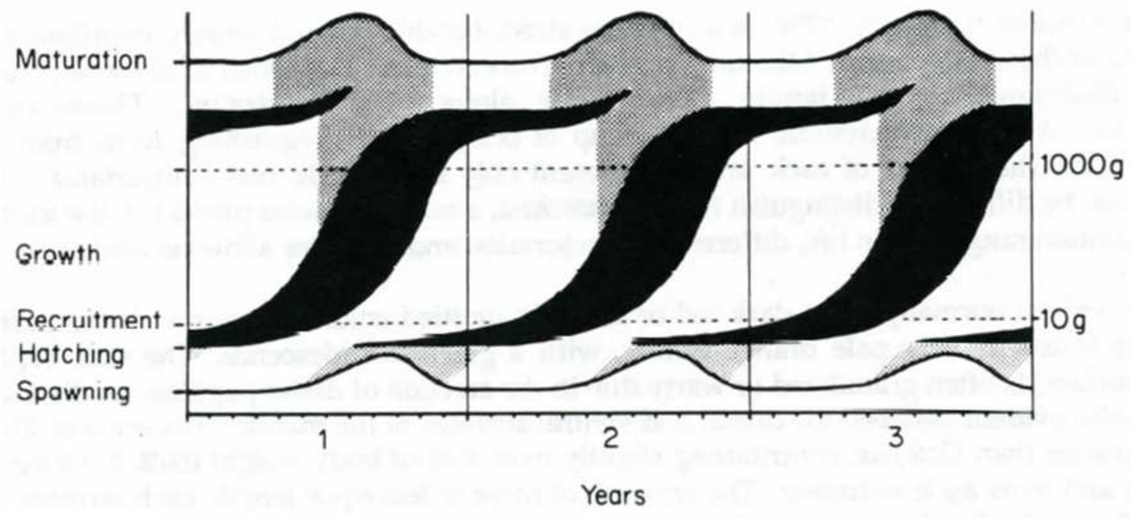


Figure 2.7. Composite model of the life cycle of *Eledone cirrhosa* proposed by Boyle (1983) based mainly on data from the northern population. The estimated period of peak spawning activity is shown on the lower line. Growth (solid black) can proceed at widely differing rates up to recruitment and during the first year of life. Rapid growth (upper margin) and early maturity will allow individuals to contribute to the spawning population of that year (dark stipple) after which they will die at about 12-15 months old. The lower margin of the growth curve indicates a situation in which growth occurs slower and later in the season and leads to a 2-year cycle; the overwintering population matures and contributes to the early part of the spawning peak in its second year (top line). Variation in the time of spawning and the conditions for growth and maturation can lead to alternative 1- and 2-year cycles or to both life cycle modes arising within the same cohort. (After Boyle, 1983).

Distribution & Habitat

Eledone cirrhosa geographical range comprises NE Atlantic and Mediterranean Sea (Guerra, 1992) (see Figure 2.8). In the Mediterranean the species is found from the western edge of the basin (Giordano et al., 2010; Mangold-Wirz, 1963), extending towards the east in the Adriatic Sea (Casali et al., 1998), the Aegean Sea (Tursi et al., 1995) and Eastern Mediterranean (Öztürk et al., 2014). In the Atlantic the species is distributed following the continental shelf off the coast of the Iberian Peninsula (Guerra, 1992), through the English Channel and British Isles (Rees, 1956; Stephen, 1944) and the North Sea, up to the western coast of Norway (Grieg, 1933). *E. cirrhosa* is also present in southern Iceland and Hebrides. Its southern distribution limit is located at a still undefined region in NW African coast.

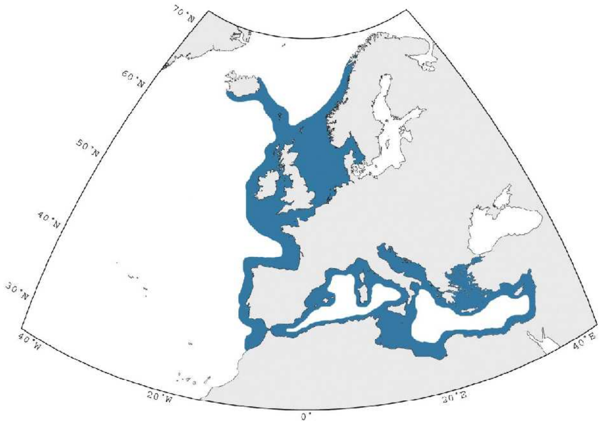


Figure 2.8. Distribution range of *Eledone cirrhosa* (After Belcari *et al.*, 2015).

Eledone cirrhosa is a typical benthic species which inhabits on a wide range of habitats throughout its distribution range, from sand and mud to broken-rock and rocky reefs, and from coastline to continental shelf and slope. A number of ecological, morphological and reproductive differences have been pointed between specimens from the North Atlantic and the Mediterranean (Boyle, 1997). Thus, published information indicates differences in the variety of habitats it occupies, body size and several reproductive parameters (Table 2.3).

This species shows a wide bathymetric distribution, although it seems to be different within its range. Thus, in Western Mediterranean it lives on sandy and muddy bottoms from 25 m up to 630 m deep (Wurtz et al., 1992), although the most significant concentrations of individuals occur between 50 and 300 m depth (Belcari et al., 2002; Mangold-Wirz, 1963). On the other hand, it is known that in the North Atlantic this species spreads its bathymetric range, reaching coastal areas. Specifically in Scottish waters its presence has been reported by scuba divers (Boyle, 1983b). Its deepest record is around 1000 m in Hebrides slope (Boyle et al., 1998).

Literature usually indicates a bias in the relative abundance of both sexes, suggesting segregation by sex or/and size-age classes (Boyle and Knobloch, 1982; Mangold-Wirz, 1963; Moriyasu, 1981). Boyle (1997) noted that the sex ratio was 1:1 in deep waters of the Mediterranean Sea, but also noticed a bias towards females in shallower waters during springtime. Based on the sex ratio and maturity stages at different depth strata, Boyle pointed out that higher abundances of mature females present in shallower waters during summer was a trace of the existence of a reproductive migration event. This type of indirect observation is an example of the kind of clues in which many life history traits have been inferred, mainly in those species inhabiting deep-waters, since animals hardly tolerate tagging and therefore the release-recapture practices are not feasible.

Table 2.3. Main ecological and reproductive characteristics in North Atlantic and Western Mediterranean *Eledone cirrhosa* populations.

	North Atlantic (Boyle, 1983)	**Western Mediterranean (Mangold-Wirtz, 1963)**
Maximum Weight	2,000 g	Less than 1,000 g
Oocytes number	2,200-55,000	Up to 3,900
Oocytes size	7 mm (mode)	1.8-7 mm
Spermatophore number	97-290	Up to 150
Spermatophore size	36-49 mm	43-54 mm
Reproductive period	Throughout the year	End of may to mid-august
Bathymetric distribution	Shoreline – 1,000 m(*)	50-300 m
Habitat	Rock, sand and mud	Muddy bottoms

(***** Boyle, 1998)

Despite the species follows a general trend in its distributional pattern, a more complex spatio-temporal pattern arises on a smaller geographical scale and the species seems to display a patchy distribution. Size and sex-based segregated distribution and seasonal reproductive migrations make difficult to compare data from different areas, since this migratory event do not occur at once in different locations. The available information on its depth distribution is mainly based on scientific surveys that usually are undertaken over a precise time period and do not cover the entire potential bathymetric range and as a rule operate up to a certain depth. Therefore, the presence of *E. cirrhosa* in shallower waters could have passed unnoticed.

Given the high commercial importance of this species in the Mediterranean Sea, several studies have been made about its distribution and abundance in this area. Belcari et al. (2002) analysed the distribution, abundance and demographic structure of this species and *E. moschata* throughout the whole Mediterranean Basin, obtained from surveys performed from late spring to midsummer, from 1994 to 1999. According to their findings, *E. cirrhosa* is a very abundant species in the western basin and was locally very abundant in the northern Ligurian and Tyrrhenian seas, as well as in the northern Adriatic. In the central area of the Mediterranean its abundance decreases to increase again eastwards. It is interesting to note that the sampling intensity in the Western Mediterranean was considerably higher.

Studies addressing a smaller geographical scope have revealed local distribution and life history particularities. Thus, surveys undertaken in the Eastern Mediterranean (Tracian and Aegean Sea) indicate that *E. cirrhosa* is more abundant in the northern area (Salman et al., 2000). During summer most of the individuals were adults while the fishings performed in autumn were mostly composed by recruits (Mantle Length or ML 20-60 mm) comprising a higher proportion of the catch (Lefkaditou and Papaconstantinou, 1995). Instead, in autumn nearly all of the catch was comprised of juveniles below 50 mm ML and large individuals of the summer completely disappeared. In this area the reproduction of *E. cirrhosa* seems to start in early summer and last till mid of autumn (Salman et al., 2002; Tursi et al., 1995).

The horned octopus lives at depths from 25 to 400 m in the Adriatic Sea, but it is most abundant over 75 m. The species is less abundant than *E. moschata* in the central and Northern Adriatic, while in the south the situation is the opposite (Casali et al., 1998).

Trawling surveys undertaken in the northwestern Mediterranean (Ligurian Sea) for 14 months between 20 and 90 m depth showed that *E. cirrhosa* appear from 30 m depth onwards and juveniles mainly appeared at deeper strata (-90 m) (Relini and Orsi Relini, 1984). The adults dissapeared from July onwards. In this context, circalitoral level was suggested to constitute or to be part of a nursery area, moving to unlocated deeper areas for spawn. Southwards, in the Tyrrenian Sea, where *E. cirrhosa* is the most abundant cephalopod species (Belcari and Sartor, 1993), high inter-annual variations in the abundance were observed during autumn (Giordano et al., 2006), the species was present at all bathymetric strata (comprising from 10 to 800m depth), becoming rarer below 50 m and beyond 500 m depth (Giordano et al., 2010). The highest densities concentrated between 100 and 200 m depth, while the biomass was highest between 50 and 100 m depth. In this season, both recruits and adults were well represented in all bathymetric strata. Recruit and adult groups were not well separated, with very small recruits (25-30 mm ML) located at a depth of 100m depth, while larger specimens (up to 150 mm ML) were found at 200-500 m. The adult group showed a polymodal pattern in all strata. During spring and summer higher densities were found between 50 and 200 m depth (Wurtz et al., 1992). Mature males preferred deeper bottoms than mature females at all seasons. Based on the presence of juveniles, recruitment in this area has been suggested to be progressively delayed later in the seasom from north to south.

In Portugal the species is heterogeneously distributed throughout the coast (Cabido, 1984), inhabiting rocky, sandy and muddy bottoms between 32 and 480 m depth, but preferably between 60 and 80 m, below which adult specimens were rarely found.

Available data on *E. cirrhosa* distribution in Galician waters indicate higher abundances occurring between 100-300 m depth (Pérez-Gándaras et al., 1980), although, since individuals were not sexed in these surveys, it was not possible to define migrations or segregation behavior by sex. Comparisons between results in different years indicated that recruitment in this area takes place in late-summer (Pérez-Gándaras et al., 1980).

The aforementioned studies point out the high spatio-temporal variability in abundance recorded for the species. In addition to the inherent interannual variability in cephalopods, several studies propose that observed population are strongly linked with the climatic fluctuations associated with climate change, declines correlating directly with increasing seawater temperatures (e.g. for *E. cirrhosa* in the Ligurian Sea (Jereb et al., 2014)). On the other hand, as abovementioned, the inverse relationship between the abundances of both species of the genus was reported in certain areas (Belcari et al., 2002) as well as with *O. vulgaris* (Relini and Orsi Relini, 1984). This fact indicates the lack of information regarding interspecific relationships (either by competition, predator / prey relationships, etc.), which together with abiotic factors would affect abundance and distribution patterns of organisms in the environment they share, and which generally they are barely considered, either by the difficulty of their study or complexity for modelling.

Trophic ecology

Cephalopods possess unique adaptations for prey catching and handling, allowing them for hunting a wide variety of preys. They have a ring of 8-10 arms with which they can immobilize relatively large prey compared with same-sized fishes. Another set of adaptations, such as possessing a highly developed sensory system and the cryptic capacity, based in the combined action of diverse epithelial and muscular cells which allow them not only change colour, but also texture, makes them masters of stalking and camouflage. On the other hand, their muscular bodies and highly energetic tissues make them a very tempting prey for larger predators. So that, many marine animals prey on *E. cirrhosa*. Marine mammals such as harbour seals (*Phoca vitulina*) (Brown and Pierce, 1997; Tollit et al., 1998) have been noted as major predators of *E. cirrhosa* and some small cetaceans, such as Rhisso’s dolphin (*Grampus griseus*) and long-finned pilot whales (*Globicephala melas*), are almost exclusively teuthophagous and *E. cirrhosa* has been pointed as a main prey for these species in Scottish (MacLeod et al., 2014) and Galician waters (González et al., 1994) or Western Mediterranean (Blanco et al., 2006). Moreover, *E. cirrhosa* has also been pointed as prey of fish, both osteichthyes (Salman and Karakulak, 2009) and chondrichthyes, such as rays (Mulas et al., 2015) or sharks (Clarke and Stevens, 1974). *E. cirrhosa* has also been cited as prey of other cephalopods such as *Loligo forbesii* (Guerra and Rocha, 1994).

Since studies on cephalopods are mainly focused on commercially relevant, coastal living species, a large amount of knowledge gaps are present in the life history of the great majority of the species and therefore we only have knowledge of some feeding habits of 30-40% of the described cephalopod species (Nigmatullin, 2005). The diet of *E. cirrhosa* has been analised mainly in two areas along all its distribution range. Studies undertaken in the North Atlantic and the Western Mediterranean Sea highlight a common prey range throughout the geographic distribution area of the species. Overall, we can verify that the horned octopus is an active predator, which feeds mainly on benthic crustaceans, mostly alpheids and brachyurids (Boyle and Knobloch, 1981). Moreover, a variety of prey has been observed, such as fish and, in a lesser extent, molluscs, echinoderms, polychaeta and cephalopod eggs (Auteri et al., 1988; Ezzeddine et al., 2012; Sánchez, 1981). Cannibalism has also been recorded (Guerra, 1992; Moriyasu, 1981).

Until recently, most studies on cephalopod diet were invariably based on visual identification of stomach contents. *E. cirrhosa* has been cited in the North Atlantic as a significant predator of such commercially important species as *Homarus gammarus* (European lobster), *Nephrops norvegicus* (Norway lobster), and *Cancer pagurus* (edible crab) from traps (Boyle, 1986b). Nevertheless, these kinds of large prey are not reported in the available literature, which can be due to limitations in prey identification techniques. In cephalopods, as the oesophagus passes through the brain, it has very little possibility of dilatation and accordingly, food may be ingested in small pieces. This is the reason why cephalopods chop up their prey with the beak before eating them. There are, however, some material that enters into the stomach, such a scales, otholits and lenses for fishes, skeletal parts, gill lamellae and eggs from crustacean, statoliths, pens and lenses from cephalopods and setae from polychaetes. From such remains is usually possible to identify the prey, but identification is not always easy and this technique is very time consuming. This will lead in most, if not all cases, to an underestimation of some type of prey eaten, since those with little or no hard parts will go undetected. Furthermore, some octopuses perform an external digestion permitting the ingestion of soft parts of crabs without the need of taking in the hard parts as well. As shown in the section dealing with feeding strategies and diet in this PhD, other methologies have been developed to analyse stomach contents in *E. cirrhosa*, but again they are very much time consuming as well as expensive.

Fisheries

Cephalopods are an important fishery resource in European waters. Total cephalopod landings from the Northeast Atlantic and Mediterranean Sea have routinely exceeded 100 000 t since 1980 and were more than 116 000 t in 2006, comprising 49 000 t landed into Europe from the Northeast Atlantic and ca. 67 000 t landed from the Mediterranean by all nations, including those African and Asian countries with Mediterranean coastlines (Pierce et al., 2010). Nevertheless, there are significant cultural differences regarding the consumption and therefore, in the fishing effort focused on cephalopods between northern Europe and the countries of the Mediterranean Basin. Cephalopods in the Mediterranean represent a resource of great commercial value, whereas they are only important commercial species in Spain, Portugal and some French Atlantic regions considering the Atlantic Ocean. This may well be attributable to cultural factors (Sartor et al., 1998). Therefore, given that *E. cirrhosa* distribution range comprises the Mediterranean Basin and European Atlantic coasts, levels of exploitation to which this species is subjected widely vary throughout its geographic range.

A number of studies indicate the association between macroscale oceanographic and climatic events and larval survival, recruitment, distribution and migration patterns of different stocks of marine species (see Hare, 2014). Particularly, annual variability in cephalopod abundances is strongly influenced by environmental variability, although the underlying causes of the links between environment and population dynamics are poorly understood (Pierce et al., 2008). Studies relating *E. cirrhosa* landings with environmental conditions are scarce. However, the results of one of these studies showed that catches of this species in the Gulf of Lions were related with the Rhone river run-off inputs (Lloret et al., 2001). Sanchez et al. (2004) indicated that the main factor explaining variability in CPUE was the seasonal availability of the resource (ca. 40% of the variability explained). The catch per unit effort was higher in the late spring and summer months, coinciding with the annual recruitment pulse and the majority of the fishing effort was undertaken between 100 and 200 m depth. The importance of seasonal effects in this fishery was discussed in terms of the recruitment of *E. cirrhosa* and consumer preferences.

A preliminary analysis of time series of *E. cirrhosa* landings in northwestern Iberian fishing ports (Galician and North Portugal) was performed during the development of the present study. This analysis confirmed the seasonality of catches, but did not indicate a significant effect of seasonal upwelling on its temporal pattern (unpublished data).

Many cephalopod species are important as incidental catches in trawl fisheries. However, *Octopus vulgaris* and *E. cirrhosa* are target species in some areas. A high proportion of cephalopods in demersal catches from heavily fished areas, such as the Adriatic Sea, tends to suggest that their exploitation rate is high (Lleonart, 2008). The bulk of *E. cirrhosa* catches are registered in the lower continental shelf and upper slope of the northern Mediterranean and northern Aegean Sea, where it is likely the most abundant octopod species (Lefkaditou et al., 2000). Since no quotas are set in European cephalopod fisheries, deliberate misreporting or illegal landing of catches is not a major issue. However, the low taxonomic resolution of official landings statistics, the relatively high importance of poorly recorded artisanal landings, and indeed patchy reporting of commercial landings from some ports, are issues to take into account (Hastie et al., 2009). In fact, *E. cirrhosa* is generally pooled with *E. moschata* in Mediterranean fishery statistics (Lefkaditou et al., 2000), as well as in other areas (e.g. Portugal; Cabido, 1984). In any case, *E. cirrhosa* is a target species of significant economic importance in the Mediterranean Sea (Giordano et al., 2010; Orsi Relini et al., 2006; Sánchez et al., 2004), where the species is harvested on a large scale primarily by multispecific bottom trawlers and to a lesser extent with seines by small scale fisheries.

In the Western Mediterranean, fishing effort is ussually concentrated during the recruitment period, leading to the capture of young specimens (up to 3 cm LM) particularly sought for its high commercial value (Belcari and Sartor, 1999; Sánchez et al., 2004). The best catches occur between July and December (Sánchez and Martín, 1993) and usually less than 10% of catches are discarded (Sartor et al., 1998). Fishing of juveniles takes place on the continental shelf, mainly between 50 and 150 m depth. Almost the 40% of the variability in catch per unit effort (CPUE) of juvenile *E. cirrhosa* in the Catalan Sea is explained by the seasonal availability of the resource (Sánchez et al., 2004). In the Ligurian sea new recruits appear in January, although they become commercially relevant between April and October (Relini and Orsi Relini, 1984). During the 1990's the species was overexploited in the South Adriatic Sea and CPUE was dramatically reduced, although a recovering trend was observed in 2006 (Orsi Relini et al., 2006).

In northern Europe *E. cirrhosa* is considered a by-catch of trawling fisheries targeting other species of major commercial interest (mainly hake, monkfish and Norway lobster). Commercial value of *E. cirrhosa* is relatively low in that area. Both in the Mediterranean Sea and the NE Atlantic, the species is sometimes caught in crab fisheries (Relini et al. 2006), causing problems for these valuable fisheries (Boyle, 1983, 1986b). When the capture of this species is high and it is landed, much of the catches are exported to countries of the Mediterranean Basin (Relini et al. 2006).

In a time serie of landings from 2000 to 2013, *E. cirrhosa* landings in Spain ranged from 1432 t in 2003 to 578 t in 2008. Landings followed a decrease trend from 2003 to 2008 in all areas, with a slight increase at the end of the time-serie with 1148 tn in 2013 (ICES, 2014). Despite the drop of the fishing trawling fleet in some areas (e.g. Galician), this decline may be due to the increase in the depth at which trawlers operate, which has rise in recent decades. This was mainly shows by the gear marks on soft bottoms detected at depths of 200-1300 m in the NE Atlantic (Lorance, 2007; Roberts *et al.*, 2000).

Total landings of *Eledone* spp in ICES areas VIIIc and IXa in 2013 were 1453 t. Pair trawl metier accounted for 28 t in the western Bay of Biscay (VIIIc), with no significant discard. According with an ICES report, landings in NW Iberian Peninsula (Subdivision IXa north and VIIIc) provide the highest values with 1145 t, followed by 308 t in south Portugal and 177 t in the central region. Abundances of the horned octopus in ICES Subareas III, IV, V and VI are almost anecdotic compared to abundances in the rest of the ICES areas (ICES, 2014).

According to official data, landing amounts of *E. cirrhosa* for Galician fishing-trawl fleet for the period 2004 - 2015 were very variable, ranging from less than 700 t in the last two years to 2012 t in 2007 (www.pescadegalicia.com). During this period a notable decrease in the number of trawlers occurred in this area, reaching a maximum of 114 in 2007 and slightly decreasing since then until the actual 70 vessels in 2015 (Figure 2.9). These variations and other parameters have to be taken into account at the time of studying catch tendencies for exploited resources and, obviously, *E. cirrhosa* is not an exception.

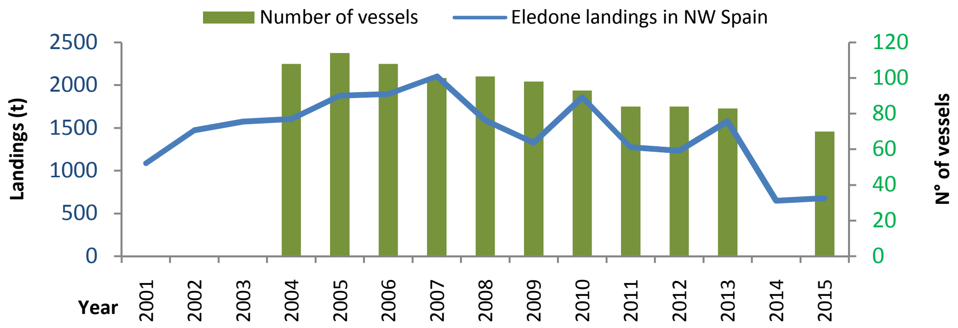


Figure 2.9. *Eledone cirrhosa* landings and number of vessels in Galicia (NW Iberian Peninsula) during the period 2001-2015.

References

Allcock, A.L., Cooke, I.R., Strugnell, J.M., 2011. What can the mitochondrial genome reveal about higher-level phylogeny of the molluscan class Cephalopoda? Zoological Journal of the Linnean Society 161, 573–586.

Allcock, A.L., Lindgren, A., Strugnell, J.M., 2015. The contribution of molecular data to our understanding of cephalopod evolution and systematics: a review. Journal of Natural History 49, 1373–1421.

Alvarez, I., Gomez-Gesteira, M., Castro, M.d., Lorenzo, M.N., Crespo, A.J.C., Dias, J.M., 2010. Comparative analysis of upwelling influence between the western and northern coast of the Iberian Peninsula. Continental Shelf Research 31, 388-399.

Arístegui, J., Barton, E.D., Álvarez-Salgado, X.A., Santos, A.M.P., Figueiras, F.G., Kifani, S., Hernández-León, S., Mason, E., Machú, E., Demarcq, H., 2009. Sub-regional ecosystem variability in the Canary Current upwelling. Progress in Oceanography 83, 33-48.

Auteri, R., Manini, P., Volpi, C., 1988. Regime alimentare di *Eledone cirrhosa* (Lamarck, 1798) e *Sepia orbignyana* (Férrussac in Orbigny, 1826) (Mollusca, Cephalopoda) nel Tirreno Settentrionale. Quaderni del Museo di Storia Naturale di Livorno 9, 67-73.

Belcari, P., Sartor, P., 1993. Bottom trawling teuthofauna of the northern Tyrrhenian Sea. Scientia Marina 57, 145-152.

Belcari, P., Sartor, P., 1999. *Eledone cirrhosa* (Lamarck, 1798), in: al., G.R.e. (Ed.), Sintesi delle conoscenze sulle risorse dap esc dei fondi del Mediterraneo centrale (Italia e Corsica). Biologia Marina Mediterranea, pp. 737-746.

Belcari, P., Tserpes, G., González, M., Lefkaditou, E., B., M., Piccinetti, G.M., Souplet, A., 2002. Distribution and abundance of *Eledone cirrhosa* (Lamarck, 1798) and *E. moschata* (Lamarck, 1798) (Cephalopoda: Octopoda) in the Mediterranean Sea. Scientia Marina 66, 143-155.

Belcari, P., Sartor, P., Jereb, P., Lefkaditou, E., Pierce, G.J., Piatkowski, W., Borges, T., Allcock, A.L., 2015. *Eledone cirrhosa*, in: Jereb, P., Allcock, A.L., Lefkaditou, E., Piatkowski, U., Hastie,L. C. Pierce,G.J. (Ed.), Cephalopod biology and fisheries in Europe: II. Species Accounts. ICES Cooperative Research Report Rapport Des Recherches Collectives pp. 30-41.

Bello, G., 2003. The biogeography of Mediterranean cephalopods. Biogeographia 24, 209-226.

Blanco, C., Raduán, M.A., Raga, J.A., 2006. Diet of Risso's dolphin (*Grampus griseus*) in the Western Mediterranean Sea. Scientia Marina 70, 407-411.

Boleda, R. M. (1976) Contribución al estudio del crecimiento y desarrollo en *Eledone cirrosa* (Lamarck, 1798). Tesina de licenciatura. Universidad de Barcelona. 54 pp.

Boyle, P.R., 1981. Methods for the aquarium maintenance of the commom octopus of British waters, *Eledone cirrhosa*. Laboratory Animals 15, 327-331.

Boyle, P., 1983a. Ventilation rates and arousal in the Octopus. Journal of Experimental Marine Biology and Ecology 69, 129-136.

Boyle, P.R., 1983b. *Eledone cirrhosa*, in: Boyle, P.R. (Ed.), Cephalopod life cycles. Vol I, Species Accounts. Academic Press, pp. 365-386.

Boyle, P., 1986a. Responses to water-borne chemicals by the octopus *Eledone cirrhosa* (Lamarck, 1798). Journal of Experimental Marine Biology and Ecology 104, 23-30.

Boyle, P.R., 1986b. A descriptive ecology of *Eledone cirrhosa* (Mollusca: Cephalopoda) in Scottish waters. Journal of the Marine Biological Association of the United Kingdom 66, 855-865.

Boyle, P.R., 1997. *Eledone cirrhosa*: biology and fisheries in the Eastern Atlantic and Mediterranean, in: Lang, M.A., Hochberg, F.G. (Eds.), Proceedings of the workshop on the fishery and market potential of *Octopus* in California. Smithsonian Institution, Washington D.C., pp. 99-103.

Boyle, P.R., Boletzky, S.v., 1996. Cephalopod Populations: Definition and Dynamics. Philosophical Transactions of the Royal Society of London

Boyle, P.R., Collins, M.A., Williamson, G.R., 1998. The cephalopod by-catch of deep water trawling on the Hebrides slope. Journal of the Marine Biological Association of the United Kingdom 78, 1023-1026.

Boyle, P.R., Chevis, D., 1992. Egg development in the octopus *Eledone cirrhosa*. Journal of Zoology 227, 623-638.

Boyle, P.R., Dubas, F., 1981. Components of body pattern displays in the Octopus *Eledone cirrhosa* (Mollusca: Cephalopoda). Marine Behavior & Physiology 8, 135-148.

Boyle, P.R., Knobloch, D., 1981. Hole boring of crustacean prey by the octopus *Eledone cirrhosa* (Mollusca: Cephalopoda). Journal of Zoology 193, 1-10.

Boyle, P.R., Knobloch, D., 1982. Sexual maturation in the octopus *Eledone cirrhosa* Lamarck. Malacologia 22, 189-196.

Boyle, P.R., Knobloch, D., 1983. The female reproductive cycle of the octopus, *Eledone cirrhosa*. Journal of the Marine Biological Association of the United Kingdom 63, 71-83.

Boyle, P.R., Knobloch, D., 1984a. Male reproductive maturity in the octopus *Eledone cirrhosa* (Cephalopoda: Octopoda). Journal of the Marine Biological Association of the United Kingdom 64, 573-579.

Boyle, P.R., Knobloch, D., 1984b. Reproductive maturity in fresh and acuarium-held *Eledone cirrhosa* (Cephalopoda: Octopoda). Journal of the Marine Biological Association of the United Kingdom 64, 581-585.

Boyle, P.R., Mangold, K., Ngoile, M.A., 1988. Biological variation in *Eledone cirrhosa* (Cephalopoda: Octopoda): Simultaneous comparison of North Sea and Mediterranean populations. Malacologia 29, 77-87.

Boyle, P.R., Thorpe, R.S., 1984. Optic gland enlargement and female gonad maturation in a population of the octopus *Eledone cirrhosa*: A multivariate analysis. Marine Biology 79, 127-132.

Boyle, P., Rodhouse, P., 2005. Cephalopods: Ecology and Fisheries, Blackwell Publishing, UK.

Brown, E.G., Pierce, G.J., 1997. Diet of harbour seals at Mousa, Sheland, during the third quarter of 1994. Journal of the Marine Biological Association of the United Kingdom 77, 539-555.

Cabido, M.T.J., 1984. Aspectos da biologia de *Eledone cirrhosa* (Lamarck, 1798), *Eledone moschata* (Lamarck, 1798) e *Octopus vulgaris* (Cuvier 1797) (Mollusca : cephalopoda) na costa portuguesa, Instituto Nacional de Investigação das Pescas. Universidade de Lisboa, Lisboa, 94 pp.

Casali, G., Piccinetti, M., Soro, S., 1998. Distribuzione di cefalopodi in Alto e Medio Adriatico. Biologia Marina Mediterranea 5, 307-318.

Clarke, M.R., 1986. A Handbook for the identification of cephalopods beaks. Oxford.

Clarke, M.R., Stevens, J.D., 1974. Cephalopods, blue sharks and migration. Journal of the Marine Biological Association of the United Kingdom 54, 949-957.

Cobb, C.S., Pope, S.K., Williamson, R., 1995a. Circadian rhythms to light-dark cycles in the lesser octopus, *Eledone cirrhosa*. Marine and Freshwater Behaviour and Physiology 26, 47-57.

Cobb, C.S., Williamson, R., Pope, S.K., 1995b. The responses of the epistellar photoreceptors to light and their effect on circadian rhythms in the lesser octopus, *Eledone cirrhosa*. Marine and Freshwater Behaviour and Physiology 26, 59-69.

Doubleday, Z., Semmens, J., Pecl, G., Jackson, G., 2006. Assessing the validity of stylets as ageing tools on *Octopus pallidus*. CIAC 2006 Hobart, Tasmania Book of Abstracts, 61.

Ezzeddine, S., Rjeibi, M., Chemmam, B., 2012. Regime alimentaire d´*Eledone cirrhosa* (Cephalopoda, Octopoda) des côtes nord et est Tunisiennes. Bulletin de l'Institut National des Sciences et Technologies de la Mer de Salammbô 39, 25-33.

Fariña Pérez, A.C., 1996. Megafauna de la plataforma continental y talud superior de Galicia. Biología de la cigala, *Nephrops norvegicus*. Tesis doctoral. Departamento de Bioloxía Animal, Bioloxía Vexetal e Ecoloxía. Universidad de La Coruña.

Fraga, F., 1987. Oceanografía de la plataforma gallega. Santiago de Compostela.

Forsythe, J. W. and Van Heukelem, W. F. 1987. Growth, in: Boyle, P.R. (Ed.), Cephalopod life cycles. Vol II, Comparative Reviews. Academic Press, pp. 135-156.

Giordano, D., Busalacchi, B., Perdichizzi, F., Perdichizzi, A., Profeta, A., Rinelli, P., Greco, S., 2006. Abundance trend of *Octopus vulgaris* and *Eledone cirrhosa* (Mollusca: Cephalopoda) in the southern Tyrrhenian Sea. Biologia Marina Mediterranea 13, 270-271.

Giordano, D., Busalacchi, B., Bottari, T., Perdichizzi, F., Profeta, A., Perdichizzi, A., Pirrera, L., Modica, L., Rinelli, P., 2010. Population dynamics and distribution of *Eledone cirrhosa* (Lamarck, 1798) in the Southern Tyrrhenian Sea (Central Mediterranean) Cahiers de Biologie Marine 51, 213-227.

González, A.F., López, A., Guerra, A., Barreiro, A., 1994. Diets of marine mammals stranded on the northwestern Spanish Atlantic coast with special reference to Cephalopoda. Fisheries Research 21.

Grieg, J.A., 1933. Cephalopods from the west coast of Norway. Bergen Mus. Arb. 1933, naturv. R. 4, 1-25.

Guerra, A., 1975. Determinación de las diferentes fases del desarrollo sexual de *Octopus vulgaris* Lamarck, mediante un índice de madurez. Investigación Pesquera 39, 397-416.

Guerra, A., 1992. Mollusca, Cephalopoda. Fauna Ibérica. Museo Nacional de Ciencias Naturales. CSIC. Madrid. 1, 327 pp.

Guerra, A., Rocha, F., 1994. The life history of *Loligo vulgaris* and *Loligo forbesi* (Cephalopoda : Loliginidae) in Galician waters (NW Spain). Fisheries Research 21, 43-69.

Hanlon, R.T., Messenger, J.B., 1996. Cephalopod Behaviour. Cambridge University Press.

Hare, J.A., 2014. The future of fisheries oceanography lies in the pursuit of multiple hypotheses. ICES Journal of Marine Science 71 2343-2356.

Hastie, L.C., Pierce, G.J., Wang, J., Bruno, I., Moreno, A., Piatkowski, U., Robin, J.P., 2009. Cephalopods in the North-Eastern Atlantic: Species, Biogeography, Ecology, Exploitation and Conservation. Oceanography and Marine Biology: Annual Review 47, 111-190.

Hayashi, Y., 1971. Estudies on the maturity condition of the common squid - III. Ponderal Index and weight indices of internal organs during maturation and exhaustion. Bulletin of the Japanese Society for the Science of Fish 37, 960-963.

Hermosilla, C.A., Rocha, F., Fiorito, G., González, Á.F., Guerra, Á., 2010. Age validation in common octopus *Octopus vulgaris* using stylet increment analysis ICES Journal of Marine Science 67, 1458–1463.

Hochberg, F.G., Nixon, M., Toll, R.B., 1992. Order Octopoda, Leach, 1818, in: Sweeney, M.J., Roper, C.F.E., Mangold, K., Clarke, M.R., Boletzky, S.v. (Eds.), “Larval” and juvenile cephalopods: a manual for their identification. Smithsonian Contributions to Zoollogy, pp. 213-279.

Isgrove, A. 1909. *Eledone*. Liverpool Marine Biological Communication, Mem. 18. London.

ICES, 2014. Report of the Working Group on Cephalopod Fisheries and Life History (WGCEPH). Lisbon, Portugal.

Jackson, G.D., 2004. Cephalopod growth: historical context and future directions. Marine and Freshwater Research 55, 327-329.

Jereb, P., Roper, C.F.E., Norman, M.D., Finn, J.K., 2014. Cephalopods of the world. An annotated and illustrated catalogue of cephalopod species known to date. Volume 3. Octopods and Vampire Squids. Rome.

Jozet-Alves, C., Darmaillacq, A.S., Boal, J.G., 2014. Navigation incephalopods, in: Darmaillacq, A.S., Dickel, L., Mather, J. (Eds.), Cephalopod cognition. Cambridge University Press.

Leftkaditou, E., Papaconstantinou, C., 1995. Distribution, growth and maturity of *Eledone cirrhosa* (Cephalopoda:Octopoda) in the Thracian Sea (Eastern Mediterranean). Rapport Commission International Mer Méditerranée, 34, 247.

Lefkaditou, E., Souplet, A., Peristeraki, N., Gonzalez, M., Kavadas, S., Vidoris, P., Cuccu, D., Papaconstantinou, C., 2000. Preliminary investigation of factors affecting the spatial distribution and abundance of *Eledone cirrhosa* (cephalopoda: octopoda) in the Mediterranean sea. In: Demersal resources in the Mediterranean Éd. Ifremer, Actes Colloq. 26, 53-63.

Lindgren, A.R., Pankey, M.S., Hochberg, F.G., Oakley, T.H., 2012. A multi-gene phylogeny of Cephalopoda supports convergent morphological evolution in association with multiple habitat shifts in the marine environment. BMC Evolutionary Biology 12, 129.

Lipinski, M., 1998. Cephalopod life cycles:patterns and exceptions. South African Journal of Marine Science 20, 439-447.

Lleonart, J., 2008. Review of the state of Mediterranean and Black Sea fishery resources, in: Basurco, B. (Ed.), The Mediterranean fisheries sector. A reference publication for the VII meeting of Ministers of agriculture and fisheries of CIHEAM member countries, Zaragoza (Spain).

Lloret, J., Lleonart, J., Solé, I., Fromentin, J.-M., 2001. Fluctuations of landings and environmental conditions in the north-western Mediterranean Sea. Fisheries oceanography 10, 33-50.

Lorance, 2007

MacLeod, C.D., Santos, M.B., Burns, F., Brownlow, A., Pierce, G.J., 2014. Can habitat modelling for the octopus Eledone cirrhosa help identify key areas for Risso’s dolphin in Scottish waters? Hydrobiologia 725, 125-136.

Mangold-Wirz, K., 1963. Biologie des Céphalopodes bentiques et nectoniques de la Mer Catalane. Vie et Milieu., 285 pp.

Mangold, K., 1987. Reproduction, in: Boyle, P.R. (Ed.), Cephalopod Life Cycles. Academic Press, pp. 157-200.

Mangold, K., Boletzky, S.v., Frösch, D., 1971. Reproductive biology and embryonic development of *Eledone cirrhosa* (Cephalopoda: Octopoda). Marine Biology 8, 109-117.

Moriyasu, M., 1981. Biologie des pêches de céphalopodes benthiques. Application aux eledones, *Eledone cirrhosa* (Lam. 1798) du golfe du Lion. Université des Sciences et Techniques du Langue-doc, France p. 225.

Moriyasu, M., Benhalima, K., 1993. Morphological and morphometrical observations on the mantle stylet of *Eledone cirrhosa* (Lamarck, 1798) (Cephalopoda: Octopoda). VENUS (Jap. Jour. Malac.) 52, 149-154.

Mulas, A., Bellordi, A., Cannas, R., Cau, A., Cuccu, D., Marongiu, M.F., Porcu, C., Follesa, M.C., 2015. Diet and feeding behaviour of longnosed skate *Dipturus oxyrinchus*. Journal of Fish Biology 86, 121-138.

Muntz, W.R.A., 1991. Anatomical and behaviour studies on vision in *Nautilus* and *Octopus*. American Malacological Bulletin 9, 69-74.

Naef, A., 1923. Die Cephalopoden. Fauna e Flora del Golfo di Napoli 35, 313-863.

Nigmatullin, C.M., 2005. Towards the correct methodology of cephalopod feeding study: review of some neglected problems. III Simposio Internacional sobre calamares del Pacífico y II Taller Internacional sobre calamares, Lima, Perú Libro de resúmenes, 3 pp.

Nixon, M. 1998. Overview of Cephalopod Characters. In: Voss, N. A., Vecchione, M. , Toll, R. B., Sweeney, M. J. Systematics and Biogeography of Cephalopods. Smithsonian Contributions to Zoology, 586: 7-9.

Nixon, M., Mangold, K., 1996. The early life of *Octopus vulgaris* (Cephalopoda: Octopodidae) in the plankton and at settlement: a change in lifestyle. Journal of Zoology 239, 301-327.

Nixon, M., Young, J.Z., 2003. The brains and lives of cephalopods. Oxford University Press. New York.

O'Dor, R. K., Shadwick, R. E., 1989. Squid, the Olympian cephalopods. Journal of cephalopod biology 1(1): 33-55.

Orelli, M.v., 1962. Die Übertragung der Spermatophore von Octopus vulgaris und *Eledone* (Cephalopoda). Revue suisse de Zoologie 69, 193-202.

Orsi Relini, L., Mannini, A., Fiorentino, F., Palandri, G., Relini, G., 2006. Biology and fishery of *Eledone cirrhosa* in the Ligurian Sea. Fisheries Research 78, 72-88.

Otero, P., Ruíz-Villarreal, M., Peliz, A., Cabanas, J.M., 2010. Climatology and reconstruction of runoff time series in northwest Iberia: influence in the shelf buoyancy budget off Ría de Vigo. Scientia Marina 74, 247-266.

Öztürk, B., Doğan, A., Bitlis-Bakir, B., Salman, A., 2014. Marine molluscs of the Turkish coasts: an updated checklist. Turkish Journal of Zoology 38, 832-879.

Pérez-Gándaras, G., 1983. Estudio de los cefalópodos ibéricos: Sistemática y bionomía mediante el estudio morfométrico comparado de sus mandíbulas, Departamento de Zoología y Biología Animal. Facultad de ciencias biológicas. Universidad Complutense de Madrid, Madrid, p. 318.

Pérez-Gándaras, G., Guerra, A., Vázquez, A., Alonso-Allende, J.M., Labarta, E., Fuertes, J.R., López-Veiga, E.C., 1980. Distribución y abundancia de especies demersales de Galicia, Informes técnicos del Instituto de Investigaciones Pesqueras. CSIC, Barcelona.

Pierce, G.J., Allcock, L., Bruno, I., B., P., González, Á., Guerra, Á., Jereb, P., al., e., 2010. Cephalopod biology and fisheries in Europe. ICES Cooperative Research Report No. 303.

Pierce, G.J., Valavanis, V.D., Guerra, A., Jereb, P., Orsi-Relini, L., Bellido, J.M., Katara, I., Piatkowski, U., Pereira, J., Balguerias, E., Sobrino, I., Lefkaditou, E., Wang, J., Santurtun, M., Boyle, P.R., Hastie, L.C., MacLeod, C.D., Smith, J.M., Viana, M., González, A.F., Zuur, A.F., 2008. A review of cephalopod-environment interactions in European Seas. Hydrobiologia 612, 49-70.

Rees, W.J., 1956. Notes on the European species of *Eledone* with especial reference to eggs and larvae. Bulletin of British Museum of Natural History (Zoology) 3, 283-291.

Relini, G., Orsi Relini, L., 1984. The role of cephalopods in the inshore trawl fishing of the Ligurian Sea. Oebalia 10, 37-58.

Rey, D., Álvarez-Iglesias, P., Araújo, M.F., Bernaeu, A.M., Comas, M., Decastro, M., Druet, M., Ferreira Da Silva, E., Ferrín, A., Gesteira, M., Martins, V., Mohamed, K.J., Rubio, B., Vilas, F., 2014. The NW Iberian continental shelf, in: Chiocci, F.L.C., A. R. (eds) (Ed.), Continental Shelves of the World: Their Evolution During the Last Glacio-Eustatic Cycle.

Rey, J., 1993. Relación morfosedimentaria entre la plataforma continental de Galicia y las Rías Bajas y su evolución durante el cuaternario. Publicaciones especiales. Instituto Español de Oceanografía, 1-233.

Roberts, J. M., Harvey, S. M., Lamont, P. A., Gage, J. D., Humphery, J. D., 2000. Seabed photography, environmental assessment and evidence for deep-water trawling on the continental margin west of the Hebrides. Hydrobiologia 441:173-183

Robson, G.C., 1929. A monograph of the recent Cephalopoda. Part I. Octopodinae, London.

Robson, G.C., 1932. A monograph of the recent Cephalopoda. Part II. The Octopoda, excluding Octopodinae, London.

Rocha, F. Guerra, A. and González, A.F., 2001. A review of the reproductive strategies in cephalopods. Biological Reviews 76:291-304.

Rodríguez-Domínguez, A., Rosas, C., Méndez-Loeza, I., Markaida, U., 2013. Validation of growth increments in stylets, beaks and lenses as ageing tools in *Octopus maya*. Journal of Experimental Marine Biology and Ecology 449, 194-199.

Sacarrao, G.F., 1951. Notice on embryonic shell sac of *Octopus* and *Eledone*. Revista da Facultade de Ciencias de Lisboa 1, 371-374.

Salman, A., Katagan, T., Benli, H.A., 2002. Cephalopod fauna of the Eastern Mediterranean. Turkish Journal of Zoology 26, 47-52.

Salman, A., Karakulak, F.S., 2009. Cephalopods in the diet of albacore, *Thunnus alalunga*, from the Eastern Mediterranean. Journal of the Marine Biological Association of the United Kingdom 89, 635-640.

Salman, A., Katagan, T., Gücü, A.C., 2000. The Distribution and Fishing of two Mediterranean *Eledone* spp. (Octopoda:cephalopoda) in the Aegean Sea. Turkish Journal of Zoology 24, 165-171.

Sánchez, P., 1976. Contribución al estudio de la maduración sexual de *Eledone cirrosa*. Tesina de licenciatura. Universidad de Barcelona. 34 pp.

Sánchez, P., 1981. Regime alimentare d'*Eledone cirrhosa* (Lamarck, 1798) (Mollusca, Cephalopoda) dans la Mer Catalane. Rapport Commission International Mer Méditerranée 27, 209-212.

Sánchez, P., Maynou, F., Demestre, M., 2004. Modelling catch, effort and price in a juvenile *Eledone cirrhosa* fishery over a 10-year period. Fisheries Research 68, 319-327.

Sartor, P., Belcari, P., Carboell, A., Gonzalez, M., Quetglas, A., Sánchez, P., 1998. The importance of cephalopods to trawl fisheries in the Western Mediterranean. South African Journal of Marine Science 20, 67-72.

Smith, P.J.S., 1981. The role of venous pressure in regulation of output from the heart of the octopus, *Eledone Cirrhosa* (Lam.). Journal of Experimental Biology 93, 243-255.

Sroczynski, S., Muntz, W.R.A., 1985. Image structure in *Eledone cirrhosa*, an octopus. Zoologische Jahrbüker 89.

Stephen, A.C., 1944. The Cephalopoda of Scottish and adjacent waters. Transactions of the Royal Society of Edinburgh 61, 247-270.

Strugnell, J.M., Norman, M.D., Vecchione, M., Guzik, M., Allcock, A.L., 2014. The ink sac clouds octopod evolutionary history. Hydrobiologia 725, 215-235.

Teles-Machado, A., Peliz, A., McWilliams, J.C., Cardoso, R.M., Soares, P.M.M., Miranda, P.M.A., 2015. On the year-to-year changes of the Iberian Poleward Current. Journal of Geophysical Research: Oceans 120, 4980-4999.

Tollit, D.J., Black, A.D., Thompson, P.M., Mackay, A., Corpe, H.M., Wilson, B., Parijs, S.M.V., Grellier, K., Parlane, S., 1998. Variations in harbour seal *Phoca vitulina* diet and dive-depths in relation to foraging habitat. Journal of Zoology 244, 209-222.

Trigo, R.M., Pozo-Vázquez, D., Osborn, T.J., Castro-Díez, Y., Gámiz-Fortis, S., Esteban-Parra, M.J., 2004. North Atlantic oscillation influence on precipitation, river flow and water resources in the Iberian Peninsula. International Journal of Climatology 24, 925-944.

Tursi, A., D'Onghia, G., Lefkatidou, E., Maiorano, P., Panetta, P., 1995. Population biology of *Eledone cirrhosa* (Mollusca, Cephalopoda) in the north Aegean Sea (Eastern Mediterranean Sea). Vie et Milieu 45, 139-145.

Villanueva, R., Norman, M.D., 2008. Biology of the planktonic stages of benthic octopuses. Oceanography and Marine Biology: An Annual Review 46, 105-202.

Wells, M.J., 1978. *Octopus*: Physiology and Behaviour of an Advanced Invertebrate. Chapman and Hall, London.

Wells, M.J., 1980. Nervous control of the heartbeat in *Octopus*. Journal of Experimental Biology 85, 111-128.

Wooster, W.S., Bakun, A., Mclain, D.R., 1976. The seasonal upwelling cycle along the Eastern boundary of the North Atlantic. Journal of Marine Research, 34, 131-141.

Wurtz, M., Matricardi, G., Belcari, P., 1992. Distribution and abundance of the octopus *Eledone cirrhosa* in the Tyrrhenian Sea, Central Mediterranean. Fisheries Research 13, 53-66.

Young, R.E., Harman, R.F., 1988. "larva", "paralarva" and "subadult" in cephalopod terminology. Malacologia 29, 201-207.

CHAPTER 3. Reproductive cycle

This chapter is published as Regueira M., González A.F., Guerra Á. and Soares, A. 2013. Reproductive traits of horned octopus Eledone cirrhosa *in Atlantic Iberian waters. J. Mar. Biol. Ass. U.K. 93: 1641-1652.*

Reproductive traits of horned octopus *Eledone cirrhosa* in Atlantic Iberian waters

Abstract/Sumary

Length-weight relationships, sex ratio, maturity patterns, size at first maturity, reproductive outputs, fecundity and spawning period of horned octopus *Eledone cirrhosa* in northwest Iberian coast are presented for the first time. Samples were collected between February 2009 and July 2011 in four fishing ports along the northwest Iberian coast: Burela in north Galician waters (NGW), Ribeira or Bueu in western Galician waters (WGW), and Aveiro in western Portuguese waters (WPW). A total of 4,127 individuals (1,042 males, 3,079 females and 6 undetermined) were sampled. Individuals ranged from 45 to 191 mm dorsal mantle length (ML) and 15 to 1159 g body weight (BW). The overall sex ratio was biased towards females, accounting 74.71% of whole sample. The spawning season of this species in northwestern Iberian waters extends from May to July. Size at first maturity ($ML_{50\%}$) of males was 108.9 mm in NGW, 99.25 mm in WGW, and 91.4 mm in WPW, whereas $ML_{50\%}$ for females were 134.5 mm, 121.4 mm and 100.8 mm, respectively. Potential fecundity was 2,452.88±36.4 oocytes per ovary (n=697). Mean oocyte length was 3.89±0.025 mm (n=697). The average number of fully developed spermatophores in mature males was 86.55±1.9 (n=223) with a mean length of 44.97±0.29 mm (n= 224). Potential fecundity in females was significantly ($p < 0.05$) correlated with ML and BW. Results obtained suggest that energy for gonad growth comes from the diet rather than endogenous reserves.

Keywords: *Eledone cirrhosa*, Reproductive biology, Maturation, NW Iberian waters.

Introduction

Eledone cirrhosa (Lamarck, 1798) is a benthic, medium-sized octopus widely distributed over the north-eastern Atlantic and Mediterranean Sea (Boyle, 1983). The Atlantic distribution of this species extends from 66-67°N (Grieg, 1933; Massy, 1928) to the Moroccan coast (Guerra, 1992). Several studies have focused on reproductive aspects of this species, especially on populations inhabiting Mediterranean sea (Lefkaditou and Papaconstantinou, 1995; Mangold-Wirz, 1963; Moriyasu, 1988), where *E. cirrhosa* has high commercial importance, and Scottish waters (Boyle and Knobloch, 1982b; Kelly, 1993a). However, their populations off the Atlantic Iberian coasts have hardly been studied.

Eledone cirrhosa in northwestern Iberian waters is a by-catch of trawling fishery, mainly focused on other species like hake, monkfish, mackerel or Norway lobster. Despite of it, *E. cirrhosa* is a species with increasing commercial interest, whose landings during the period 1997-2010 ranged between 545 and 2,100 metric tons only in Galicia (NW Spain) (www.pescadegalicia.com) and there are not previous studies about the populations dynamics of the species in this area. Catches in the Portuguese waters reach some 179 metric tons from 2006 to 2010 (MAMP official data).

Western coasts of the Iberian Peninsula represent the northern boundary of the NW Africa coastal upwelling system that associates with the Canary Current (Alvarez-Salgado *et al.*, 2000), driven by prevailing winds from the north in summer (upwelling) and southerly winds during the rest of the year (downwelling). Summer upwelling drives to the coast colder nutrient-rich deeper water known as Eastern North Atlantic Central Water (ENACW). This phenomenon results in a seasonal and geographic fluctuation in ocean-climatic conditions, which may influence over spawning, embryonic development, hatching, growth, recruitment, maturation and migration of cephalopods, and especially during their critical early life stages (González *et al.*, 2005; Otero *et al.*, 2009; Otero *et al.*, 2008; Pierce *et al.*, 2008).

Previous publications indicated that *E. cirrhosa* shows appreciable differences throughout its distribution range, both from morphometric and reproductive biology perspectives. Specimens from Mediterranean Sea reach almost half sizes than their conspecifics in the North Atlantic (Boyle *et al.*, 1988). Bathymetric distribution is also different, since the species displays a wide bathymetric distribution in the Mediterranean basin, generally inhabiting waters over 700 m (Belcari *et al.*, 2002), occurring most abundantly between 60-120 m (Mangold-Wirz, 1963). On the contrary, *E. cirrhosa* from the North Sea lives from shoreline of rocky coasts down to 200 m (Boyle, 1983), although it has been recorded in Faeroes at 770 m (Massy, 1928), its deeper register. Size differences do not only refer to its range, but also to sexual dimorphism, with females reaching larger sizes than males.

Sexual maturation takes place at a wide range of body sizes. Previous publications pointed out that a range of oocytes lengths are usually present in maturing and mature ovaries, and that mean oocyte length is well correlated with ovary weight and ovary index (Boyle and Knobloch, 1983). Weight of male reproductive organs is strongly correlated with body size (Boyle and Knobloch, 1982b). Experiences by Boyle and Knobloch (1983) with females from the northeastern Atlantic showed an ovary enlargement at almost any time of the year but mean ovary index of the population had a pronounced seasonal fluctuation with a period of major occurrence of mature females from July to September. In western Mediterranean, breeding season extends between March and August (Mangold-Wirz, 1963). Estimates of oocytes number in mature ovaries also showed differences between Atlantic and Mediterranean populations. Thus, potential fecundity in the Mediterranean Sea ranged from 1,250 to 5,600 oocytes (Mangold-Wirz, 1963), while the potential fecundity of *E. cirrhosa* in the Atlantic ranged from 2,200 to 55,000 oocytes, with a mean of almost 11,000 (Boyle and Knobloch, 1983).

The aim of this paper is to fulfill the existing gap in biological studies on the reproductive biology of *E. cirrhosa* in north-west Iberian populations. Data on the length-weight relationship, sex ratio, maturity patterns, spawning season, length and weight at first maturity, reproductive outputs and gonadic and condition indices for the species in that area are also presented herein for the first time.

Material and methods

Commercial lots of specimens of *E. cirrhosa* from trawling commercial vessels were monthly collected from February 2009 to January 2011 in Burela (northern Galician Waters, NGW) and Bueu or Ribeira, (western Galician waters, WGW). Moreover, animals sampled in Aveiro (western Portuguese waters, WPW) were caught from March 2010 to July 2011 (Figure 3.1). A total of 4127 animals were studied.

All animals were kept frozen at -20°C until further examination. After defrosted at room temperature, all individuals were weighed, measured and sexed. Total length (TL) and dorsal mantle length (ML) were measured to the nearest 0.01 cm, and body weight (BW) and eviscerated body weight (EBW) were taken to the nearest 0.1 g. Macroscopic maturity scale of Inejih (2000) was adapted to assign an specific maturity stage to each individual. For males this scale was as follows: I: immature, II: maturing, III: pre-set, with some spermatophores in Needham's sac, and IV: mature, fully developed spermatophore, and for females: I: immature, II: maturing, III: pre-spawning, IV: Mature and V: Post-spawning.

Whenever possible, a subsample of 30 individuals of each sex and sample site was randomly selected every month for detailed analysis of reproductive cycle. Gonad weight (GW), testis weight (TW), Needham complex weight (NCW) and spermatophoric sac weight (SSW) were recorded for males. Gonad weight (GW), ovary weight (OW), oviducal complex weight (OCW) and oviducal glands diameter (OGD) were obtained from females. All weights were taken to the nearest 0.001 g and OGD was measured to the nearest 0.1mm.

Mature males (stages IV) and pre-spawning/mature females (stages III and IV) gonads were stored in 70% ethanol and 4% formalin, respectively. These samples were used to assess fecundity. Potential fecundity (PF) in stages III and IV females was estimated by counting the number of oocytes of all sizes present in a known mass of ovary taken from ovary surface, from the ovary core and from intermediate layer, according to Laptikhovsky (2000), and extrapolating to the whole ovary mass. Average length of oocytes was inferred by taking at random sample of 25 oocytes from the ovary and then measuring to the nearest 0.01 mm. Relative fecundity was calculated as the ratio of PF and BW. Concerning to males, all the spermatophores present inside the Needham sac were counted in mature ones. The length of all spermatophores was measured to the nearest 0.01 mm.

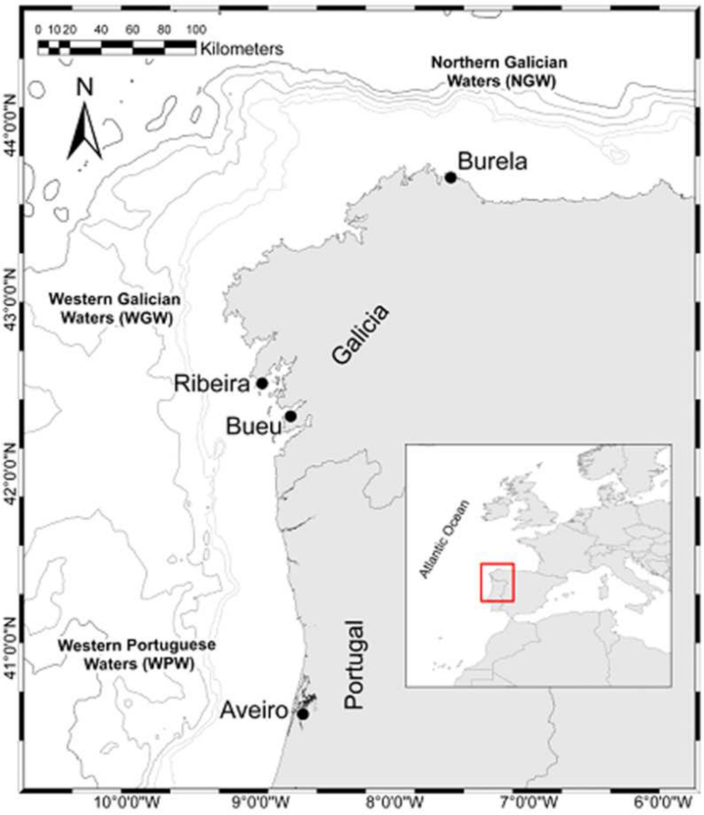


Figure 3.1. North west iberian waters showing the sampling sites in north Galician waters (NGW), west Galician waters (WGW) and west Portuguese waters (WPW).

Digestive gland weight (DGW) was obtained to the nearest 0.01 g in order to calculate the digestive gland index (DGI = (DGW/(BW-DGW)) * 100) for assessing condition state (Castro *et al.*, 1992). With the purpose of check if energy for gonadic maturation comes from feeding or from somatic tissue, three regressions were performed for males and females in each coastal side, using ML as independent variable and GW, EBW and DGW as the dependent variables, following methodology described by McGrath (2004). Relationships between standardized residuals were analyzed, so that if, for example, energy for gonad growth would be obtained at the expense of muscle tissues, a negative correlation between ML-GW and ML-EBW residual pairs would be expected.

Length-weight relationships were performed for males and females in different seasons and maturity stages by fitting the data to the equation $BW=a*ML^b$ using ordinary least square method. The value of *b* expresses the allometry level. Significant differences from 3 (isometry) were evaluated by linear transformation of the potential equation ($y=a*x^b \Rightarrow \log y = \log a+b*\log x$), calculating confidence interval of *b* and then checking whether value 3 was within the range.

Reproductive cycle was assessed by monthly progression of sex ratio (females : males), macroscopic maturity stages and also by the variation of the following indexes: Gonadosomatic index (GSI): GSI_m = (TW / BW-TW) * 100; GSI_f = (OW / (BW -OW) * 100, and Hayashi index following Guerra (1975): HI_m =NCW/ (NCW + TW); HI_f =OCW/(OCW + OW).

Mature males and females (stage IV) were used to calculate the size-at-maturity ($ML_{50\%}$) and the percentage of mature individuals per month. $ML_{50\%}$ was estimated by fitting relative length-frequency distribution for 10 mm mantle length classes to a logistic curve with the formula: $P_i=1/1+\exp[-\alpha+\beta ML_i)]$, where $ML_{50\%} =-\alpha/\beta$ (Sifner and Vrgoc, 2009). Non-linear least squares fitting method was used. Weight at 50% maturity ($BW_{50\%}$) was calculated following the same method.

Pearson (r) and Spearman-rank correlation (S_r), in the case of non-normal data, were used for evaluating covariation between variables. Differences in variables among categorical predictors were tested using non-parametric Kruskal-Wallis test for multiple comparisons and Mann-Whitney for paired comparisons, because assumptions of normality and homocedasticity were not fulfilled. Follow-up tests were conducted to evaluate pairwise differences among the three groups, controlling for Type I error across tests by adjusting of alpha using the Bonferroni approach (α_t). All data were treated with the statistical software STATISTICA 6.0.

Results

A total of 4,127 individuals (1,042 males, 3,079 females and 6 indeterminate) were analyzed during the present study. The specimens sampled were geographically distributed as follows: 1,271 individuals (885 females, 383 males and 3 undetermined) in NGW; 2,072 individuals (1,482 females, 588 males and 2 undetermined) in WGW, and 783 individuals (712 females, 71 males and 1 indeterminate) in WPW. Due to the high predominance of females in all sampled sites and samples, the number of males in certain months did not reach the intended 30 individuals. In these cases, all available males were analyzed.

Length-weight relationships

Size and weight data of individuals sampled by sex, season, maturity stage and sampling sites are showed in Table 3.1. Kruskal-Wallis tests showed differences in size comparing separately males and females through all three sites ($p< 0.05$). Mann-Whitney U tests showed in each site significant differences in BW and ML between males and females ($p< \alpha_t$).

Given this size differences between males and females, equations were calculated for each sex and sampling area (Table 3.2). Values of b ranged from 2.29 to 2.76 and were always significantly lower than 3 for these fittings and also for both sexes in all seasons and maturity stage (data not showed), indicating overall negative allometric growth. Highest values of b were obtained for females in all cases.

Sex ratio

Overall sex ratio (females: males) was significantly biased to females, representing 74.71% of the whole sample. Sex ratio was also biased to females through sampling zones. Sex ratio was 2.31:1, 2.52:1, and 10.02:1, for NGW, WGW and WPW, respectively. Comparison between sex ratio through sampling location showed significant differences between Galician and Portuguese samples ($p<\alpha_t$). General percentages of males and females are showed in Figure 3.2. Females predominated over males in all samples with the exception of February-March 2009 and December 2010 in WGW. Nevertheless, in these cases the ratio was close to 1:1 and there was no found a predominance of males over females.

Maturity stages, spawning season and Size and weight at first maturity

A total of 2,576 specimens (833 males and 1,743 females) were used for these analyses. Size and weight of mature males (stage IV) varied from 70 to 158 mm ML and 77 to 634 g

Table 3.1. Number, size and weight data of individuals sampled by sex, season and maturity stage by sampling site.

Season/Maturity	Sex	NGW					WGW					WPW				
		n	**ML Range (mm)**	**ML±SE**	**BW Range (g)**	**BW±SE**	**n**	**ML Range (mm)**	**ML±SE**	**BW Range (g)**	**BW±SE**	**n**	**ML Range (mm)**	**ML±SE**	**BW Range (g)**	**BW±SE**
General	F	885	69-191	124.5±0.66	77-1093	412.61±6.04	1482	45-175	106.48±0.54	15-1159	286.72±4.09	712	55-160	101.43±0.69	43-775	228.97±4.7
	M	383	74-154	108.16±0.71	79-634	257.28±4.66	588	56-158	97.29±0.75	40-634	210.12±4.61	71	50-139	91.48±1.63	42-362	144.14±6.74
Autumn	F	273	85-173	128.41±1.06	109-941	450.54±9.92	168	60-175	124.12±1.73	58-1159	449.41±15.24	156	55-145	84.86±1.21	45-743	138.22±6.82
	M	119	79-148	115.29±1.18	107-537	297.17±8.02	143	57-158	108.01±1.84	40-634	290.91±11.98	17	50-95	79.88±2.65	42-150	107.82±7.42
Spring	F	286	75-191	125.25±1.07	138-1093	401.51±9.04	495	45-167	106.32±0.85	36-994	274.13±6.04	305	65-157	107.69±0.91	68-753	262.48±6.58
	M	107	75-132	101±1.2	98-436	211.86±6.88	90	62-120	87.28±1.41	46-381	155.77±7.12	33	68-117	95±1.93	78-251	150.36±8.2
Summer	F	148	80-175	115.4±1.64	113-915	315.36±12.32	296	48-165	105.47±1.24	15-782	277.88±9.05	37	80-131	97.38±1.87	98-378	181.24±10.51
	M	89	85-139	105.9±1.15	110-394	225.79±6.08	143	62-131	103.42±1.14	41-476	230.2±7.41	0	-	-	-	-
Winter	F	178	69-180	124.86±1.66	77-1090	453.33±17.06	523	54-162	101.57±0.8	49-936	251.36±5.69	214	60-160	105.28±1.23	43-775	255.62±9.39
	M	68	74-154	109.9±1.86	79-634	300.18±13.43	212	56-140	90.17±0.99	44-530	165.15±5.22	21	80-139	95.33±3.2	80-362	163.76±16.02
Inmature	F	200	69-152	108.85±1.04	77-832	271.71±7.77	470	45-150	95.21±0.87	15-704	209.98±5.61	282	55-135	87.73±0.87	43-476	147.94±4.39
	M	49	79-127	104.45±1.46	112-335	205.69±8.14	155	57-128	93.9±1.38	40-431	181.34±7.42	26	50-105	82.81±2.18	42-226	114.04±7.54
Maturing	F	205	73-167	124.24±1.24	104-850	410.23±10.51	406	60-163	105.68±1.01	48-770	281.99±7.65	192	79-155	105.21±0.96	75-743	247.24±7.41
	M	138	75-140	109.82±1.09	98-443	257.43±6.16	148	58-138	100.09±1.59	44-476	229.88±9.65	7	68-105	89.29±4.55	78-209	130.57±17.95
Prespawning	F	198	75-175	125.21±1.36	138-1035	429.68±12.96	264	73-175	112.81±1.09	67-1159	329.52±9.7	137	80-155	110.07±1.29	98-764	286.39±11.46
	M	54	74-143	107.24±2.39	79-518	264.89±16.09	162	65-145	96.87±1.4	69-621	203.63±8.59	9	84-105	92.22±2.7	94-222	143.78±15.59
Mature	F	280	100-191	135.59±0.97	200-1093	504.46±10.39	327	75-167	119.46±0.87	120-994	373.39±8.36	100	94-160	120.92±1.28	148-775	344.13±12.24
	M	142	75-154	108.18±1.21	110-634	272.05±8.35	110	70-158	101.57±1.61	77-634	243.75±11.61	29	78-139	99.55±2.43	86-362	174.52±11.43

BW, respectively. ML and BW in females (stages III and IV) ranged from 73 to 191 mm and BW from 67 to 1159 g, respectively. Monthly evolution of the maturity stages percentage for males and females in sampling sites are showed in Figure 3.3. Fully mature females were present in NGW and WGW almost throughout the year. However, they disappeared from September to March in WPW. A peak of mature females appeared in May-June. Immature females appeared immediately after mature peak and were present during winter in all sites and increasing their proportion to the south.

Table 3.2. Length-weight fitting adjustment for males and females in NGW, WGW and WPW.

	Males	**Females**
North Galician waters (NGW)	$BW = 0.0036*ML^{2.3791}$ $n=382$; $r^2 = 0.7517$	$BW= 0.0014*ML^{2.5993}$ $n=880$; $r^2 = 0.8402$
West Galician waters (WGW)	$BW = 0.0012*ML^{2.6109}$ $n=575$; $r^2 = 0.8446$	$BW = 0.0007*ML^{2.7609}$ $n=1465$; $r^2 = 0.8867$
West Portuguese waters (WPW)	$BW=0.0043*ML^{2.2951}$ $n=71$; $r^2=0.8471$	$BW=0.0007*ML^{2.7307}$ $n=712$; $r^2=0.8828$

In WPW the predominance of immature specimens extends from August to February. Post-spawning females were not found in any of the sampled locations.

Mature males were present from early winter to late summer, exhibiting a wider maturation season, with a peak in spring. Males matured at smaller sizes than females. Concerning to WPW males, data are clearly biased in April 2010, due to the scarce number of males found in this sample (n=2), but seems to follow the same seasonal pattern than in northern samples. As in females, proportion of immature males increased towards the south.

After reaching the peak of abundance of mature individuals, stages II and III specimens almost disappear for one or two months in which the population consists in a decreasing proportion of mature individuals and a sudden growing proportion of immature

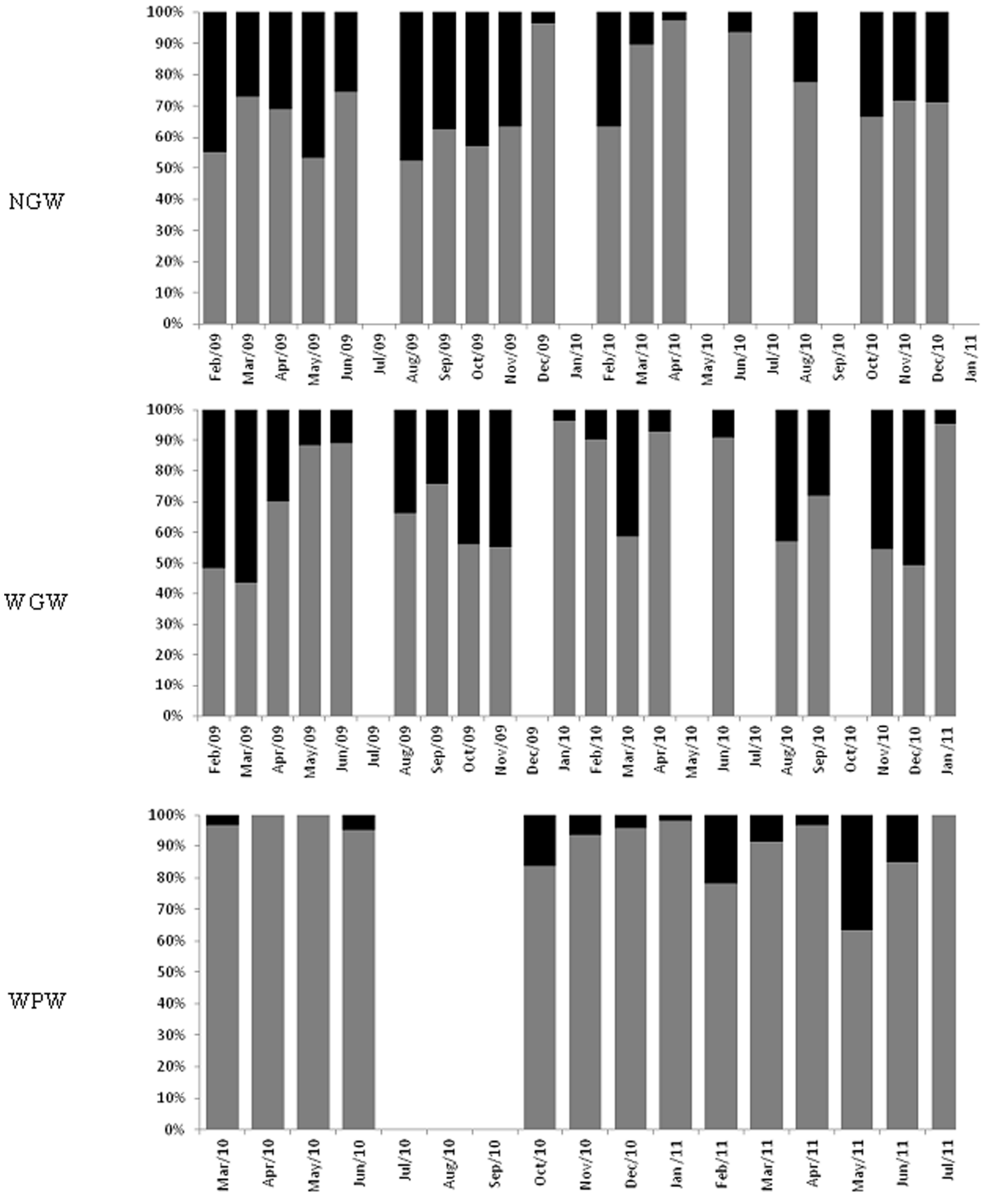


Figure 3.2. Sex ratio temporal variation in NGW(performed from Feb/09 until Dec/10), WGW (performed from Feb/09 until Jan/11) and WPW(performed from Mar/10 until Jul/11). Black and grey areas show proportion of males and females, respectively.

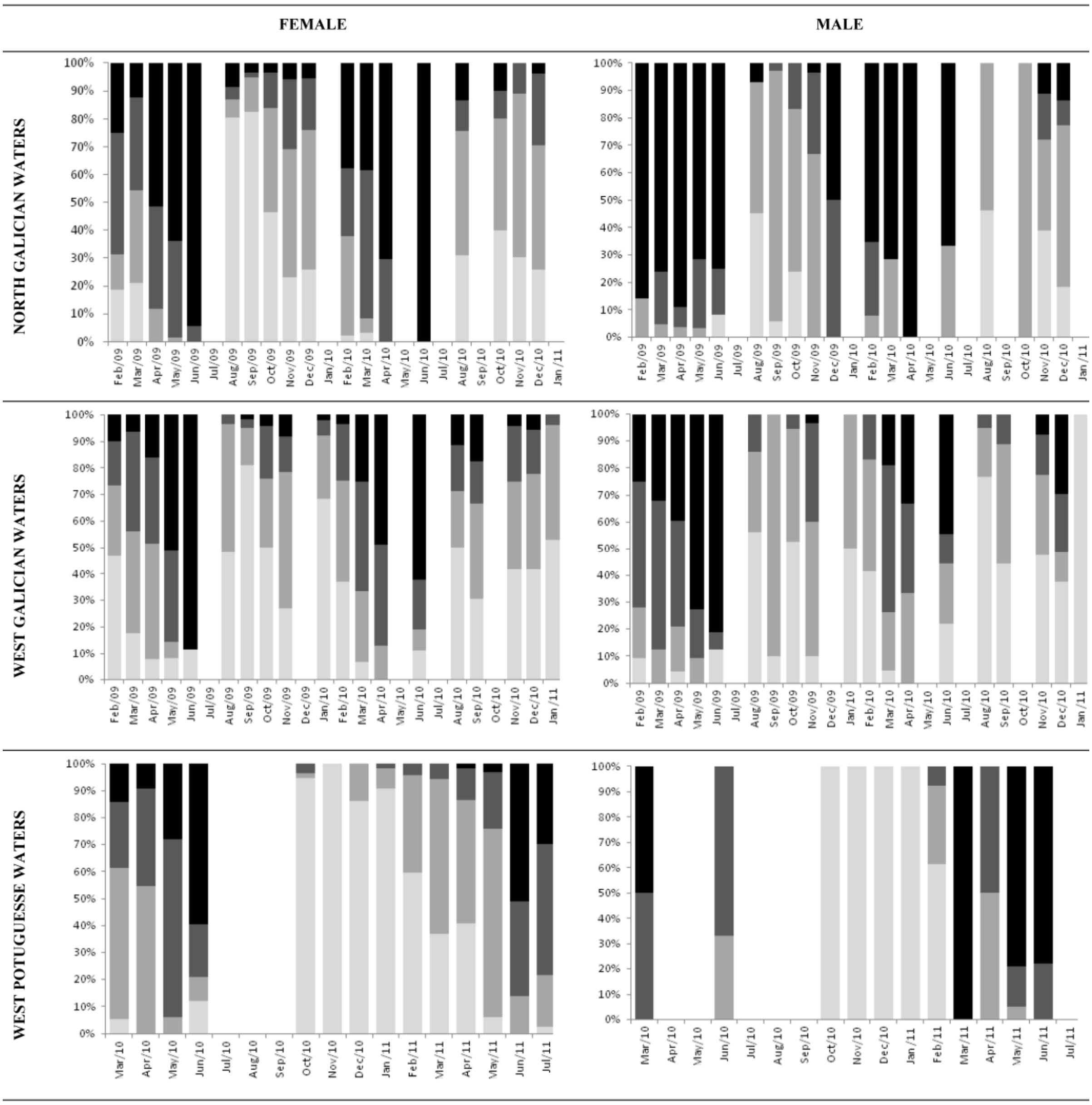


Figure 3.3. Temporal variation in Maturity stages for males and females for NGW (Feb/09 until Dec/10), WGW(Feb/09 until Jan/11) and WPW (Mar/10 until Jul/11). Increasing grey scale showing maturity stages. Lighter grey: stage I (immature); intermediate grey: stage II (maturing); dark grey: Stage III (pre-spawning); black: Stage IV (mature individuals).

Logistic fit coefficients and estimations of $ML_{50\%}$ and $BW_{50\%}$ for each sex and sampling site are showed in Table 3.3. Both $ML_{50\%}$ and $BW_{50\%}$ increase from the south towards northern waters, for both males and females.

Table 3.3. Logistic fit parameters and $ML_{50\%}$ and $BW_{50\%}$ for males and females in NGW, WGW and WPW.

	NGW		**WGW**		**WPW**	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**
α	-13.778	-14.975	-11.162	-12.423	-10.914	-10.438
β	0.126	0.111	0.112	0.102	0.119	0.103
r	0.9996	0.9995	0.990	0.9989	0.98863	0.99382
ML50% (mm)	**108.893**	**134.515**	**99.250**	**121.434**	**91.409**	**100.799**
α	-4.962	-4.916	-2.255	-4.132	-4.426	-2.775
β	0.017	0.010	0.010	0.010	0.028	0.0115
r	0.9871	0.9889	0.9659	0.9771	0.9628	0.9561
BW50% (g)	**294.303**	**497.719**	**227.486**	**415.856**	**156.757**	**242.038**

Reproductive outputs and potential/relative fecundity

Mantle length (ML) and Gonad Weight showed a significantly correlation in both males (r_s: 0.73; $p<0.05$) and females (r_s: 0.63; $p<0.05$).

Overall number of spermatophores for mature males ranged from 26 to 158, with an average of 86.55±1.89 (mean±S.E.). Spermatophore length ranged from 23.07 to 72.71 mm, with an average of 44.97±0.29 mm. The overall number of spermatophores per gram of BW in males ranged from 0.08 to 1.5, with a mean of 0.39±0.22 spermatophores g^{-1}. Table 3.4 shows the overall number of spermatophores and their sizes, as well as the number of spermatophores per gram of BW in each sampling location.

Significant differences were found (Mann-Whitney U tests; $p<\alpha_t$) in the number of spermatophores among the three sampled zones, and also concerning to their size, except among WGW and WPW. A positive correlation was found between ML and spermatophore length in mature males (r_s: 0.48; $p<0.05$; see Figure 3.4), but not between ML and spermatophore number ($p>0.05$).

Potential fecundity in pre-spawning and mature females ranged from 547 to 6545 oocytes per ovary, with an average of 2453±36. Length of oocytes ranged from 1.83 to 5.78 mm with a mean of 3.89±0.03 mm. Overall relative fecundity (RF) in whole sampled females ranged from 2.57 to 17.67 oocytes per gram, with a mean of 6.58±0.13 oocytes per gram. PF, RF and oocytes sizes in each sampling site are showed in table 3.4.

Positive correlation between PF and ML (r_s: 0.63; $p<0.05$) was found, and also between oocytes length and ML, although it was quite weak (r_s: 0.21; $p<0.05$) (Figure 3.4). PF increased with latitude, showing significant differences (Mann-Whitney U tests; $p<0.05$) among sampling sites, but oocytes sizes did not.

Eight females (0.26% of total females) were fertilized, presenting spermatangia inside the ovary. Six of them were mature individuals (stage IV), captured in winter and late spring, but two were pre-spawning animals (stage III) caught in late spring and late summer.

Table 3.4. Overall reproductive outputs and potential/relative fecundity for males and females in each sampling location.

		NGW	WGW	WPW
Males	**Spermatophore length (mm)**	46.02±0.76	44.61±0.89	41.58±0.97
	Spermatophore length range (mm)	23.07-72.71	29.78-66.87	27.119-50.58
	Spermatophore number	90.52±2.37	72.28±2.92	106.55±5.5
	Spermatophore number range	38-158	26-134	39-154
	Spermatophore per gram	0.35±0.01	0.35±0.02	0.67±0.05
	Spermatophore per gram range	0.11-0.86	0.08-0.89	0.19-1.5
Females	**Oocytes length (mm)**	3.92±0.04	3.84±0.04	3.91±0.06
	Oocytes lenght range (mm)	2.09-5.78	1.85-5.77	0.09-5.56
	Potential fecundity	2756.71±58.29	2378.75±54	1966.86±66.42
	Potential fecundity range	961-6545	547-5897	777-4802
	Relative fecundity	6.33±0.12	6.81±0.13	6.71±0.14
	Relative fecundity range	2.6-13.93	2.58-17.67	3.14-11.65

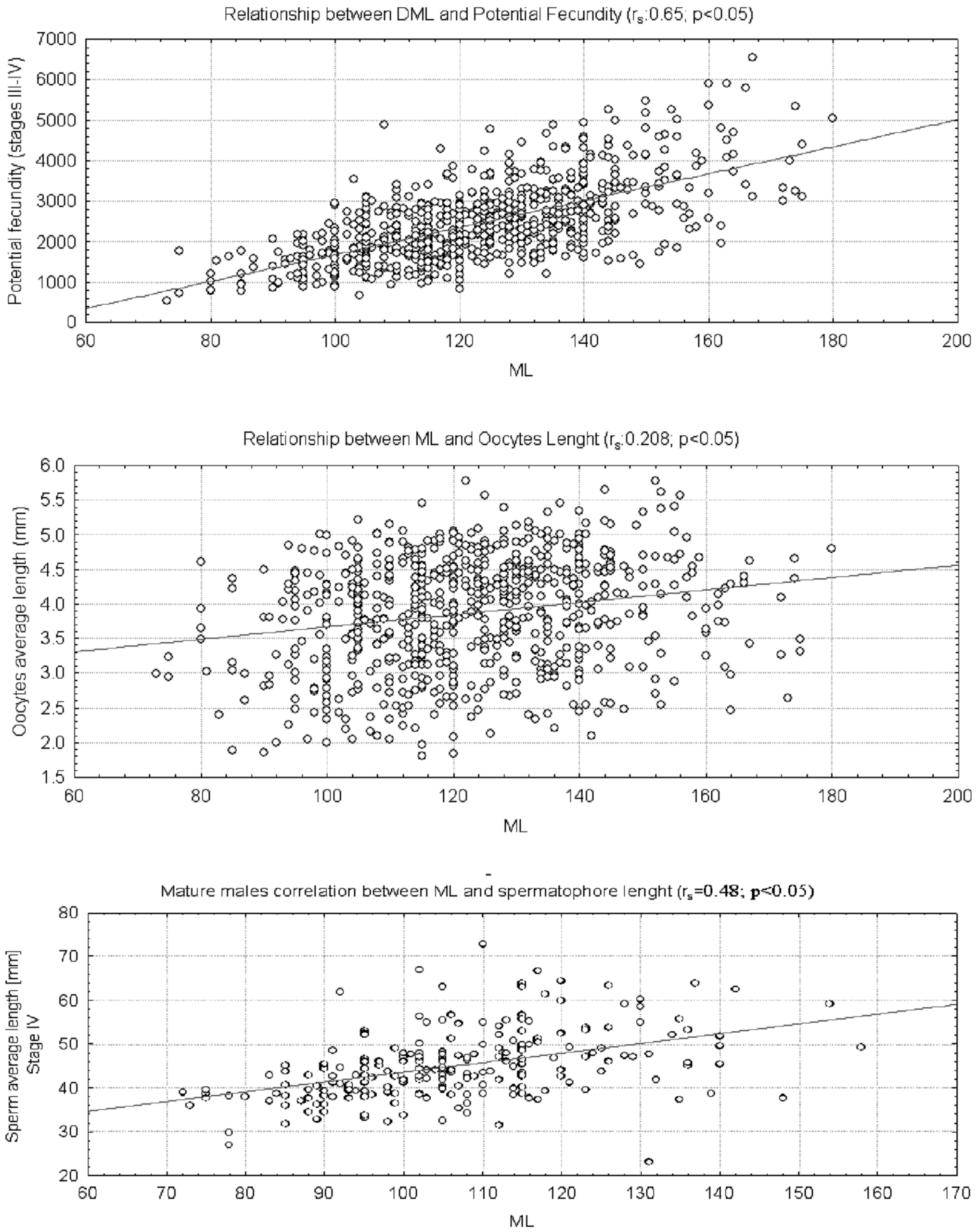


Figure 3.4. Relationships between Potential fecundity (PF), oocites size and spermatophores size versus mantle length (ML).

Gonadal and Condition indexes GSI and HI

Monthly evolution of GSI, HI and DGI by sex are showed in Figure 3.5. Gonadic indexes trends in females were similar in NGW, WGW and WPW, showing a negative overall correlation between GSI and HI (r_s=-0,54; p<0.05). Lower values of HI correspond with high maturity and vice versa. GSI reach higher values in NGW than in WGW or WPW females.

Digestive gland index (DGI) in females had an annual maximum during the spring and summer, showing significant positive correlation with GSI (r_s=0,12; p<0.05), and also with ML, BW, and maturity stage; and negative correlation with HI (r_s=-0.10; p<0.05).

Temporal evolution of male gonadic indexes (GSI and HI) followed a parallel pattern, showing significant positive correlation (r_s = 0.96, p <0.05) and negative with DGI (r_s=-0.60 and r_s=-0.55, respectively) (p <0.05). Males DGI also showed negative correlation with ML, BW and maturity stage (p<0.05).

Table 3.5. Standardized residual correlations parameters.

	Sampling site	Valid	Spearman	t(N-2)	p-level
Female GWres vs. Female EBWres	Overall	1716	-0,107	-4,459	p<0.001
	NGW	584	-0.217	-5.380	p<0.001
	WGW	643	-0.043	-1.113	0.265
	WPW	489	0.061	1.356	0.175
Female GWres vs. Female DGWres	Overall	1716	0,236	10,056	p<0.001
	NGW	584	0.255	6.364	p<0.001
	WGW	643	0.212	5.508	p<0.001
	WPW	489	0.337	7-916	p<0.001
Male GWres vs. Male EBWres	Overall	821	0,468	15,172	p<0.05
	NGW	325	0.567	12.377	p<0.001
	WGW	426	0.444	10.214	p<0.001
	WPW	70	0.087	0.718	0.475
Male GWres vs. Male DGWres	Overall	821	-0,084	-2,417	p<0.05
	NGW	325	-0.089	-1.603	0.110
	WGW	426	-0.059	-1.218	0.223
	WPW	70	-0.169	-1.416	0.161

Overall standardized residual analyses parameters are showed in table 3.5. Correlation between GW-ML residuals and DGW-ML residuals was significant and positive for females of each coastal side, suggesting that increment of gonad condition was not acquired at the expense of digestive gland reserves. Results for the analysis between GW-ML residuals and EBW-ML residuals showed weak negative correlation for NGW specimens and no correlation in WGW and WPW. Concerning to males, no significant correlation was found between GW-ML residuals and DGW-ML residuals. Analysis between GW-ML and EBW-ML standardized residuals showed positive correlation in NGW and WGW sampling sites. WPW data analysis showed no correlation.

Discussion

Comparing geographical areas, males and females *E. cirrhosa* inhabiting north-western Iberian waters were of intermediate body size on average when compared with their conspecifics of the same sex of Scottish waters, where females reach up to 2 kg BW, and the smaller animals from the western Mediterranean (Boyle *et al.*, 1988). This gradient was also observed in our study, where Portuguese individuals reached smaller sizes than their northern neighbours. Sampling bias, due to some differences in gears selectivity and fishing techniques, may influence the explanation of that gradient. However, as indicated by Boyle et al. (1988) when compared Aberdeen (Scotland) and Banyuls (western Mediterranean) populations, it is highly unlikely that these differences result from selective sampling. In our case (Galician and Portuguese waters), it is also more plausible because fishing operations are undertaken in a very similar manner in both geographical zones, since several trawlers fished in a wide area of comparable bottom topography and depths, and with the same mesh size. Size differences between animals of the same cephalopod species from different geographic areas were presented in several occasions. Thus, the squid *Loligo forbesii* from Azores differs markedly from those collected in continental shelf European Waters (Pierce *et al.*, 1994). Although body dimensions were correlated with average sea-surface, these authors indicate that it is unlikely that size differences can be accounted for simply in terms of the effect of water temperature and that it remains possible that the response to environmental factors is under genetic control. On the other hand, distinct and significant geographical clines in microsatellite allele frequencies (Perez-Losada *et al.*, 2002) and allozyme alleles (Perez-Losada *et al.*, 1999) were observed extending between the Atlantic and Mediterranean regions sampled within the range of *Sepia officinalis.* The most commonly proposed causes of clinal patterns in gene frequencies are selection across an environmental gradient, random genetic drift with isolation-by distance effects, and secondary contact and introgression between previously isolated and genetically divergent populations (Perez-Losada *et al.*, 2002).

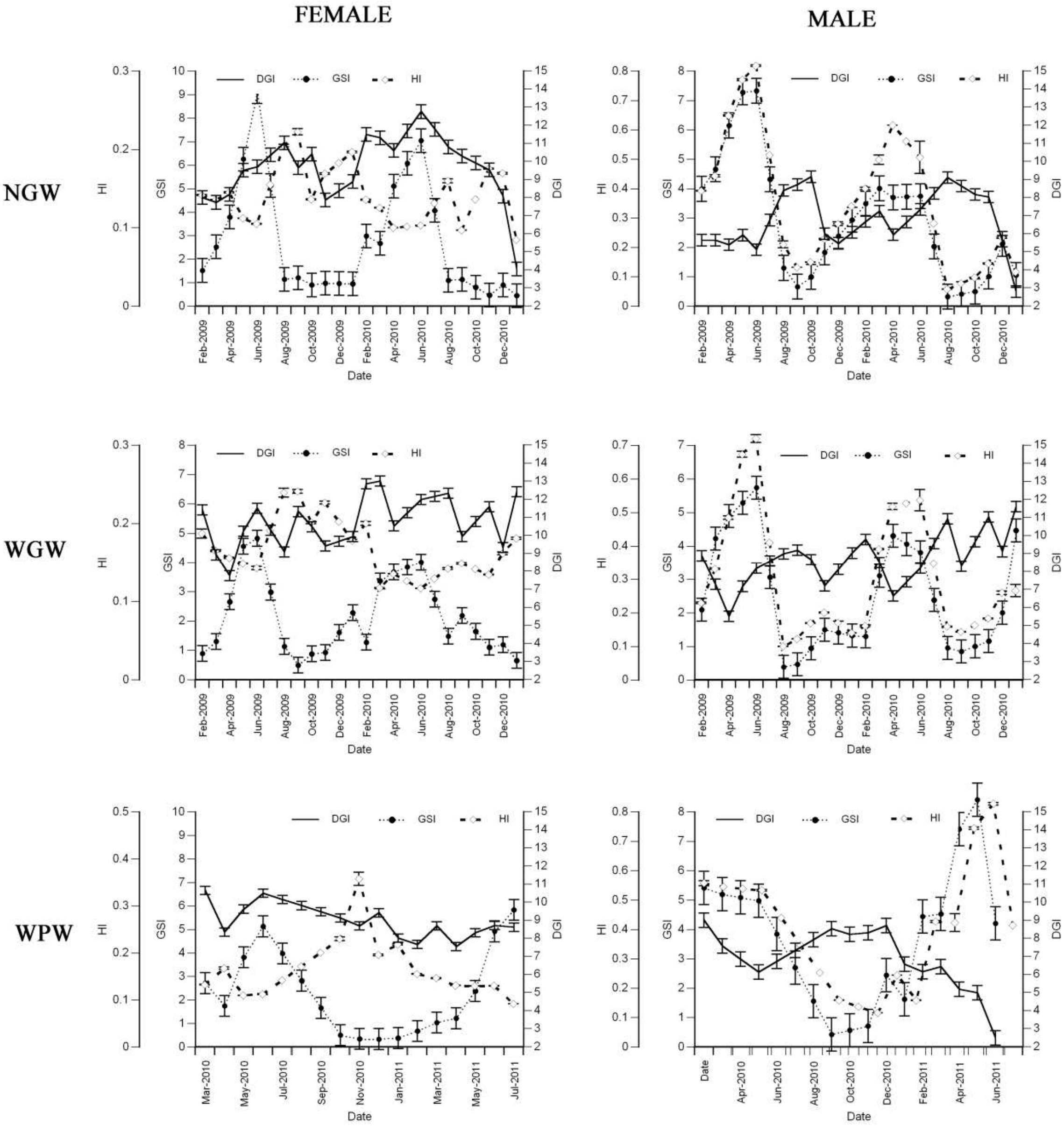


Figure 3.5. Temporal variation of Gonadal (GSI and HI) and Condition indexes (DGI) for males and females in NGW, WGW and WPW.

Values of *b* coefficient in length-weight relationship fitted were significantly lower than 3, indicating an overall negative allometric growth for males and females through all the sampling sites. Our results agreed with the observed by Marano (1993, 1996) for *E. cirrhosa* and were similar to those found in *Eledone moschata* by Sifner and Vrgoc (2009), both of them with animals from the Adriatic Sea.

Bias in sex ratio similar to that found by us in this study, mightily biased towards females, was previously reported for this species in Scottish (Boyle and Knobloch, 1982a) and western Mediterranean populations (Mangold-Wirz, 1963; Moriyasu, 1981) and in a simultaneous comparison undertaken in both areas (Boyle *et al.*, 1988). Since *E. cirrhosa* distribution in western Mediterranean shows a spatially segregated pattern by sex and maturity stages (Mangold *et al.*, 1971), population structure found in any samples could be biased for the area inhabiting by the individuals. Thus, Magold-Wirz (1963) found that males dominated over females during the reproduction period. *E. moschata* also showed male dominance in Tunisian waters (Ezzeddine-Najai, 1997) as well as in the Adriatic Sea during summer time (Sifner and Vrgoc, 2009). However, Silva et al. (2004) found that females of this species dominated significantly throughout a sampling period of one year in the Gulf of Cádiz. Fishing ground selection by commercial trawlers, which varies throughout the year depending on target species, depth, substrate composition and distance from the coastline, could account for the variation in sex ratio found. Furthermore, the smaller size of males would naturally cause them to be under-represented in trawl-caught samples (Boyle *et al.*, 1988). In any case, the lack of detailed data on the specific catch location of commercial trawlers prevents a plausible explanation of this unbalance. On the other hand, sex ratio differences throughout the year are deeply supported if bias toward one sex occurs in all sampled areas at the same time and, in consequence, occasional differences on this matter should be considered less important to obtain an accurate pattern of sex ratio.

The evolution of maturity stages as well as the condition and gonadic indices of *E. cirrhosa* females suggest that the spawning season of the species in Atlantic Iberian is concentrated in late spring and early summer. Also there were no clear differences in the reproductive season between different sampling areas. This pattern of seasonality is comparable to that described in the Mediterranean (Lefkaditou and Papaconstantinou, 1995; Mangold-Wirz, 1963; Moriyasu, 1988), but occur more earlier than the spawning season found in Scottish waters (Boyle and Knobloch, 1983), where it extends from July to September. Data about the spawning season of the congener *E. moschata* in the Gulf of Cádiz (Silva *et al.*, 2004) and the Adriatic Sea (Sifner and Vrgoc, 2009), covering from March to August, suggests that latitude, through day-night duration, is a important parameter influencing sexual maturation. Furthermore, previous publication point relationship between optic glands enlargement and female gonad maturation in *E. cirrhosa* (Boyle and Thorpe, 1984), suggesting that light perception might be involved in maturation process. Our data shows an increasing proportion of immature individuals southward in all sampling sites and for both males and females, suggesting a latitudinal effects over life-cycle, as has been suggested for other cephalopods in eastern Atlantic like *Loligo forbesii* (Boyle *et al.*, 2004; Thomas *et al.*, 2004) or *Ilex coindetii* (Arvanitidis *et al.*, 2002).

This is the first time that size and weight at first maturity is determined for *E. cirrhosa* in Atlantic waters. Samples from NGW and WGW showed larger sizes at first maturity than WPW. Portuguese samples showed a $ML_{50\%}$ similar to those reported by Soro & Piccinetti (1989) in the Adriatic Sea. These results also match in a general scenario of latitudinal gradient of variation.

In terms of reproductive parameters, previous literature indicated important differences in the number of oocytes in mature females from Atlantic and Mediterranean samplings (Boyle *et al.*, 1988). In North Atlantic populations, ovaries from mature females have more and larger oocytes than those belonging to Mediterranean samples, although Boyle (1983) suggested that those differences could be due to real differences in populations or to the methods of estimation. Concerning males, the number of spermatophores is also higher in populations from northern Europe. However, their size range is similar and even larger in individuals from the Mediterranean (Boyle and Knobloch, 1984). Differences found in spermatophore number and size studied herein, as well as the positive correlation found between ML and spermatophore length, agreed with previous publications for this species (Boyle and Knobloch, 1982b, 1984). No correlation was found between ML and spermatophore number ($p>0.05$).

The range of the number of oocytes (potential fecundity) given by Boyle & Knobloch (1983) was a 5 times higher than the estimated in the present paper. Nevertheless, it seems that PF in Scotland is higher than that in Iberian waters, as shown in Table 3.6.

Since GSI represents the percentage of ovary weight on the total weight of the individual, higher values in northern samples (Figure 3.3) might be due to a higher percentage of mature individuals northwards, unbalancing the average.

Considerable debate exists over the role of the digestive gland as a storage organ and its use for ripeness in cephalopods. Available information on this subject is often sketchy and even opposite. Somatic tissues have been found to show structural-biochemical changes. *Octopus mimus* shows decreased weight of the digestive gland and muscle mass during the spawning and egg care phases (Zamora and Olivares, 2004). However the absence of post-spawning data in *E.cirrhosa* prevents us to discuss this subject. Obtained data on the relationships between the gonadosomatic and Hayashi indices versus DGI indicate that the energy needed for the gonad ripeness in females come from the diet instead that from endogenous reserves, suggesting that digestive gland would not act as a storage organ in this specie. Our data are inconclusive about the use of muscle tissues for gonadic ripeness in females; nevertheless, relationships between standardized residuals from regressions suggest no gonadic growth at the expense of digestive gland tissues. This conclusion agrees with Rosa et al. (2004b) for *E. cirrhosa* and *E. moschata* and also with reports on other octopods, like *O. vulgaris* and *O. defilippi*, that use energy from food rather than from stored products for egg production (Rosa *et al.*, 2004a). This model contrasts with that proposed by O´Dor & Wells (1978), according to which *O. vulgaris* body musculature would be the energy supplier for final stages of maturation. Other publications support the hypothesis that digestive gland would not acts as a storage organ, like Castro et al. (1991) in *Sepia officinalis* or Semmens (1998) in loliginid squids *Sepioteuthis lessoniana* and *Photololigo sp.*

Females DGI annual maximum during spring and summer observed in our data was also found in *Octopus vulgaris* off the Galician coasts (Otero *et al.*, 2007) matching with the reproduction period and with seasonal upwelling phenomenon occurring during the summer months. The negative correlation found between GSI and HI versus DGI in males seems to indicate an opposite scenario. However, further and more detailed analyses are needed to clarify that issue, on which very few data exists in the literature.

Table 3.6. General reproductive and ecological traits of *E. cirrhosa* from three different studied areas.

	North Atlantic (Boyle, 1983)	Western Iberian peninsula	Western Mediterranean (Mangold-Wirtz, 1963)
Maximum Weight	2,000 g	1,160 g	Less than 1,000 g
Oocytes number	2200-55000	547-6545	Up to 3900
Oocytes size	7 mm (mode)	0.09-5.78 mm	1.8-7 mm
Spermatophore number	97-290	26-158	Up to 150
Spermatophore size	36-49 mm	23-73 mm	43-54 mm
Reproductive period	Throughout the year	Throughout the year, with a peak in May-June	End of may to mid-august
Bathymetric distribution	Shoreline – 770 m	55-536 m (Costa et al. 2005)	50-300 m
Habitat	Rock, sand and mud	Rock, sand and mud	Muddy bottoms

Acknowledgments

This research was supported by the project “Impacto do ambiente sobre o polbo *Eledone cirrhosa* no sistema de afloramento costeiro das augas de Galicia” (INCITE08PXIB402074PR), funded by Xunta de Galicia. We thank M. Ferreira and J. V. Vingada for their helpful to get Portuguese Samples and M.T. Fernández and M.E. García for technical assistance during the sampling. Marcos Regueira was supported by a FCT grant. We also acknowledge the improvements made by the editor and the two referees, which largely improved the paper.

References

Alvarez-Salgado, X. A., Gago, J., Miguez, B. M., Gilcoto, M. and Perez, F. F. (2000). Surface Waters of the NW Iberian Margin: Upwelling on the shelf versus Outwelling of Upwelled Waters from th Rias Baixas. *Estuarine, Coastal and Shelf Science* 51: 821-837.

Arvanitidis, C., Koutsoubas, D., Robin, J.-P., Pereira, J., Moreno, A., Cunha, M. M., Valavanis, V. and Eleftheriou, A. (2002). A comparison of the fishery biology of three *Illex coindetii* Venary, 1839 (Cephalopoda: Ommastraephidae) populations from the European Atlantic and Mediterranean waters. *Bulletin of Marine Science* 71 (1): 129-146.

Belcari, P., Tserpes, G., González, M., Lefkaditou, E., B., M., Piccinetti, G. M. and Souplet, A. (2002). Distribution and abundance of *Eledone cirrhosa* (Lamarck, 1798) and *E.moschata* (Lamarck, 1798) (Cephalopoda: Octopoda) in the Mediterranean Sea. *Scientia Marina* 66 (2): 143-155.

Boyle, P. R. and Knobloch, D. (1982a). Sexual maturation in the octopus *Eledone cirrhosa* Lamarck. *Malacologia* 22(1-2): 189-196.

Boyle, P. R. and Knobloch, D. (1982b). On growth of the Octopus *Eledone cirrhosa*. *Journal of the Marine Biological Association of the United Kingdom* 62: 277-296.

Boyle, P. R. (1983). *Eledone cirrhosa*. In: P. R. Boyle (Ed.). *Cephalopod life cycles*. Academic Press. pp. 365-386.

Boyle, P. R. and Knobloch, D. (1983). The female reproductive cycle of the octopus, *Eledone cirrhosa*. *Journal of the Marine Biological Association of the United Kingdom* 63 (1): 71-83.

Boyle, P. R. and Knobloch, D. (1984). Male reproductive maturity in the octopus *Eledone cirrhosa* (Cephalopoda: Octopoda). *Journal of the Marine Biological Association of the United Kingdom* 64: 573-579.

Boyle, P. R. and Thorpe, R. S. (1984). Optic gland enlargement and female gonad maturation in a population of the octopus *Eledone cirrhosa*: A multivariate analysis. *Marine Biology* 79 (2): 127-132.

Boyle, P. R., Mangold, K. and Ngoile, M. A. (1988). Biological variation in *Eledone cirrhosa* (Cephalopoda: Octopoda): Simultaneous comparison of North Sea and Mediterranean populations. *Malacología* 29 (1): 77-87.

Boyle, P. R., Pierce, G. J., Hastie, L. C., Wang, J., Santos, M. B., Robin, J. P. and Jereb, P. (2004). Review of variation in the life-cycle of *Loligo forbesi* across its range. *International Council for the Exploration of the Sea (CM papers and reports) 2004/CC: 32*. Poster.

Castro, B. G., Guerra, A. and Jardon, C. M. F. (1991). Variations in digestive gland weights of *Sepia officinalis* and *Sepia elegans* through their life cycles. *La Seiche, 1st International Symposium on the Cuttlefish Sepia. E. Boucaud-Camou (ed.), Centre de Publications de l'Universite de Caen*. pp. 99-104.

Castro, B. G., Garrido, J. L. and Sotelo, C. G. (1992). Changes in composition of digestive gland and mantle muscle of the cuttlefish *Sepia officinalis* during starvation. *Marine Biology* 114: 11-20.

Costa, P. R., Rosa, R., Pereira, J. and Sampayo, M. A. M. (2005). Detection of domoic acid, the amnesic shellfish toxin, in the digestive gland of *Eledone cirrhosa* and *E. moschata* (Cephalopoda, Octopoda) from the Portuguese coast. *Aquatic Living Resources* 18: 395-400.

Ezzedine-Najai, S. (1997). Maturation sexuelle d'*Eledone moschata* (Cephalopoda, Octopoda) du Golfe de Gabès (Tunisie, Méditerranée Orientale). *Vie et Milieu*. 47 (1): 69-76.

González, A. F., Otero, J., Guerra, A., Prego, R., Rocha, F. and Dale, A. W. (2005). Distribution of common octopus and common squid paralarvae in a wind-driven upwelling area (Ria of Vigo, northwestern Spain). *Journal of Plankton Research* 27 (3): 271-277.

Grieg, J. A. (1933). Cephalopods from the west coast of Norway. *Bergens Museum Aarbog*, Naturvidenskapelig rekke, 1933, *R*.4: 1-25.

Guerra, A. (1975). Determinación de las diferentes fases del desarrollo sexual de *Octopus vulgaris* Lam. mediante un índice de madurez. *Investigación Pesquera* 39(2): 397-416.

Guerra, A. (1992). Mollusca, Cephalopoda. *Fauna Ibérica. Museo Nacional de Ciencias Naturales. CSIC. Madrid.*

Inejih, C. A. (2000). Dynamique spatio-temporelle et biologie du poulpe (*Octopus vulgaris*) dans les eaux mauritaniennes: modélisation de l'abondance et aménagement des pêcheries. *Thése de Doctorat. Université de Bretagne Occidentale*. France

Kelly, K. S. (1993) *Nutrition and maturation in the octopus Eledone cirrhosa* University of Aberdeen. Master Thesis. 82 pp.

Lefkaditou, E. and Papaconstantinou, C. (1995). Distribution, growth and maturity of *Eledone cirrhosa* (Cephalopoda:Octopoda) in the Thracian Sea (Eastern Mediterranean). *Rapport Commission International pour l'exploration scientifique de la Mer Mediterranee.* 34.

MAMAOT. Ministerio da Agricultura, do Mar, do Ambiente e do Ordenamento do Territorio. http://www.portugal.gov.pt/pt/os-ministerios/ministerio-da-agricultura-mar-ambientee-ordenamento-do-territorio.aspx

Mangold-Wirz, K. (1963). Biologie des Céphalopodes bentiques et nectoniques de la Mer Catalane. *Vie et Milieu.* (13): 285 pp.

Mangold, K., Boletzky, S. v. and Frösch, D. (1971). Reproductive biology and embryonic development of *Eledone cirrhosa* (Cephalopoda: Octopoda). *Marine Biology* 8 (2): 109-117.

Marano, G. (1993) Valutazione delle risorse demersali dell’Adriatico meridionale dal promotorio del Gargano al capo d’Otranto: Relazione finale triennio ’90- ’93. *Ministero della Marina Mercantile*. Roma. (ed.) AA.VV.

Marano, G. (1996) Valutazione delle risorse demersali dell'Adriatico meridionale dal promotorio del Gargano al Capo d'Otranto: relazione finale triennio '94-'96. *Ministero per le Risorse Agricole Alimentari e Forestali*. Roma. (ed.) AA.VV.

Massy, A. L. (1928). The Cephalopoda of the Irish Coast. *Proceedings of the Royal Irish Academy* 38 Sec. B (2): 25-37.

McGrath, B.L. and Jackson, G.D. (2002). Egg production in the arrow squid *Nototodarus gouldi* (Cephalopoda: Omastrephidae), fast and furious or slow steady? *Marine Biology* 141: 699-706.

Moriyasu, M. (1981). Biologie des pêches de cephalopodes benthiques. Application aux Eledones, *Eledone cirrhosa* (Lamarck, 1798) du Golfe du Lion. *Ph.D. Dissertation, Université des Sciences et Techniques du Languedoc (France).* Ph.D. Dissertation. 225 pp.

Moriyasu, M. (1988). Analyse de la maturation sexuelle d´*Eledone cirrosa* (Cephalopoda : Octopoda) du golfe du Lion. *Aquatic Living Resources* 1: 59-65.

O'Dor, R. K. and M.J., W. (1978). Reproduction versus somatic growth: hormonal control in *Octopus vulgaris*. *Journal of experimental Biology* 77: 15-31.

Otero, J., González, A. F., Sieiro, M. P. and Guerra, A. (2007). Reproductive cycle and energy allocation of *Octopus vulgaris* in Galician waters, NE Atlantic. *Fisheries Research* 85: 122-129.

Otero, J., Alvarez-Salgado, X. A., González, A. F., Miranda, A., Groom, S. B., Cabanas, J. M., Casas, G., Wheatley, B. and Guerra, A. (2008). Bottom-up control of common octopus *Octopus vulgaris* in the Galician upwelling system, northeast Atlantic Ocean. *Marine Ecology Progress Series* 362: 181-192.

Otero, J., Alvarez-Salgado, X. A., González, A. F., Gilcoto, M. and Guerra, A. (2009). High-frequency coastal upwelling events influence *Octopus vulgaris* larval dynamics on the NW Iberian shelf. *Marine Ecology Progress Series* 386: 123-132.

Perez-Losada, M., Guerra, Á. and Sanjuan, A. (1999). Allozyme differentiation in the cuttlefish *Sepia officinalis* (Mollusca: Cephalopoda) from the NE Atlantic and Mediterranean. *Heredity* 83: 280-289.

Perez-Losada, M., Guerra, A., Carvalho, G. R., Sanjuan, A. and Shaw, P. W. (2002). Extensive population subdivision of the cuttlefish *Sepia officinalis* (Mollusca: Cephalopoda) around the Iberian Peninsula indicated by microsatellite DNA variation. *Heredity* 89: 417-424.

Pierce, G. J., Thorpe, R. S., Hastie, L. C., Brierley, A. S., Guerra, A., Boyle, P. R., Jamieson, R. and Avila, P. (1994). Geographic variation in *Loligo forbesi* in the Northeast Atlantic Ocean: analysis of morphometric data and tests of causal hypotheses. *Marine Biology* 119: 541-547.

Pierce, G. J., Valavanis, V. D., Guerra, A., Jereb, P., Orsi-Relini, L., Bellido, J. M., Katara, I., Piatkowski, U., Pereira, J., Balguerias, E., Sobrino, I., Lefkaditou, E., Wang, J., Santurtun, M., Boyle, P. R., Hastie, L.C., MacLeod, C. D., Smith, J. M., Viana, M., González, A. F. and Zuur, A. F. (2008). A review of cephalopod-environment interactions in European Seas. *Hydrobiologia* 612: 49-70.

Rosa, R., Costa, P. R. and Numes, M. L. (2004a). Effect of sexual maturation on the tissue biochemical composition of *Octopus vulgaris* y *O. defilippi* (Mollusca: Cephalopoda). *Marine Biology* 145: 563-574.

Rosa, R., Costa, P. R., Pereira, J. and M.L., N. (2004b). Biochemical dynamics of spermatogenesis and oogenesis in *Eledone cirrhosa* and *Eledone moschata* (Cephalopoda: Octopoda). *Comparative Biochemistry and Physiology, Part B* 139: 299-310.

Semmens, J. (1998). An examination of the role of the digestive gland of two loliginid squids, with respect to lipid: estorage or excretion? *Proceedings of the royal society B: Biological sciences* 265: 1685-1690.

Sifner, S. K. and Vrgoc, N. (2009). Reproductive cycle and sexual maturation of the musky octopus *Eledone moschata* (Cephalopoda: Octopodidae) in the northern and central Adriatic Sea. *Scientia Marina* 73 (3): 439-447.

Silva, L., Ramos, F. and Sobrino, I. (2004). Reproductive biology of *Eledone moschata* (Cephalopoda:Octopodidae) in the Gulf of Cádiz (south-western Spain, ICES Division IXa). *Journal of the Marine Biological Association of the United Kingdom* 84: 1221-1226.

Soro, S. and Piccinetti Manfrin, G. (1989). Biologia e pesca di Cefalopodi in Adriatico. *Nova Thalassia* 10 (1): 493-498.

Thomas, M., Challier, L., Santos, M. B., Pierce, G. J., Moreno, A., Pereira, J., Cunha, M. M., Porteiro, F., Gonçalves, J. and Robin, J.-P. (2004). Spatial differences in biological characteristics of *Loligo forbesi* (Cephalopoda Loliginidae) in the Northeast Atlantic. *International Council for the Exproration of the Sea. CM 2004/CC:23.*

Zamora, C. M. and Olivares, P. A. (2004). Variaciones bioquímicas e histológicas asociadas al evento reproductivo de la hembra de *Octopus mimus* (Mollusca:Cephalopoda). *International Journal of Morphology* 22(3): 207-216.

CHAPTER 4. Habitat and Distribution

This chapter is published as Regueira M., González A.F. and Guerra Á., 2014. Habitat selection and population spreading of the horned octopus Eledone cirrhosa *(Lamarck, 1798) in Galician waters (NW Atlantic). Fish. Res. 152: 66-73.*

Habitat selection and population spreading of the horned octopus *Eledone cirrhosa* (Lamarck, 1798) in Galician waters (NE Atlantic)

Abstract

Distribution and abundance of *Eledone cirrhosa*, a benthic octopus inhabiting the NE Atlantic and Mediterranean Sea, were studied in north-western Iberian waters. Capture data collected during two series of surveys, carried out during summer-autumn in Galician waters (NE Atlantic Ocean), were analysed. “Galicia” series (*G1, G2* and *G3*) extended along the Galician continental shelf from Ribadeo Ría to Miño Estuary in 1974, 1975 and 1976, and “Cigala” series (*C 06, C 07* and *C 08*) were recorded from Finisterre Cape to Miño Estuary in 2006, 2007 and 2008. Data from a total of 276 trawls, ranging from 50 to 500 m, and corresponding environmental parameters were transferred to a Geographic Information System (GIS), providing a platform for a spatial-temporal analysis. Bathymetric distribution, variations in abundance, biomass and average body size of the octopus *Eledone cirrhosa* were analysed through depth strata and seasons. Higher abundances were obtained in the intermediate strata and in autumn, most likely due to new recruits. Largest sizes of *E. cirrhosa* were collected in summer and average weight increased with depth throughout the sampling period. These variations of biomass and abundance suggest the existence of segregation by size and agree with the landings pattern of the trawlers fishery, in which *E. cirrhosa* is not a target species. No significant differences were found between *G1* and *G2* surveys or between *C* ones, performed at the same dates, suggesting that the polled data of surveys are useful tools for representing phenological stages of the species. Collected data and developed models illustrate a migratory behaviour during the reproductive period. Among the eight environmental variables considered, only the latitude remained significant in all developed models, suggesting an effect of photoperiod over the species distribution.

Keywords: *Eledone cirrhosa*, distribution, abundance, GIS, habitat selection, GAMs.

Introduction

The horned octopus, *Eledone cirrhosa* (Lamarck, 1798) is a benthic cephalopod inhabiting rocky and muddy bottoms over the continental shelf and slope of north-western Atlantic and Mediterranean Sea (Boyle, 1983). Its latitudinal distribution ranges from 66-67° N to an undefined limit in Moroccan coasts (Guerra, 1992).

The commercial importance of *E. cirrhosa* has increased during the last years in the Galician trawling fishery (NW Spain), which targets hake, mackerel, monkfish or Norway lobster. Its landings ranged from 545 to 2100 metric tons in Galicia during the last 15 years (www.pescadegalicia.com). Despite its increasing economic importance, its biology in north-western Iberian waters (Spain and Portugal) still remains poorly known, in contrast with the rest of its distribution range. Factors affecting the distribution of *E. cirrhosa* in the Mediterranean have been previously studied, particularly from a fishery perspective, and mainly in central and western Mediterranean basins (Belcari *et al.*, 2002; Giordano *et al.*, 2010; Lefkaditou *et al.*, 2000; Pertierra and Sánchez, 2005; Salman *et al.*, 2000; Wurtz *et al.*, 1992). On the other hand, previous studies on this species, comparing north Atlantic and Mediterranean populations, revealed significant biological (mean size, spawning season, fecundity, etc.) and ecological (bathymetric distribution, habitat selection, etc.) differences (Boyle *et al.*, 1988). Western Mediterranean specimens are smaller, with a maximum weight lower than 1 Kg, and inhabits muddy bottom in circalitoral and bathyal levels (Mangold-Wirz, 1963). Spawning season peaks from end of May to August (Mangold-Wirz, 1963), although some individuals spawn in April (Mangold *et al.*, 1971). On the other hand, north Atlantic specimens reach larger sizes (up to 2 Kg) and dwell in a wider range of depth, from coastline to continental slope (Boyle, 1983). Early life stages of this species in this area have been found throughout the year, which confirm an extended spawning season (Boyle, 1983 quoting Russel 1921 and Stephen, 1944) . In Scottish waters, *E. cirrhosa* is caught in rocky, sandy or muddy bottoms. Since this species have no commercial interest in northern Europe, most of the catches are sold in local markets and exported to Mediterranean countries (Boyle, 1986).

Landings of this species in the North-western Iberian harbours show a cyclical annual pattern (Figure 4.1), closely linked to their reproductive cycle (Regueira et al., 2013). During spring (March-May), and coinciding with the maturation peak, captures reach its maximum and then decreases until late summer (July to September), when most of adult individuals disappear due to the post-reproductive mortality. Because the continental shelf areas off Spain and Portugal are relatively narrow *E. cirrhosa* is distributed along a thin fringe parallel to the coast connecting north Atlantic and Mediterranean waters. This Iberian population shows intermediate characteristics between north Atlantic and Mediterranean populations (Regueira *et al.*, 2013), illustrating the gradient in the ecosystem conditions.

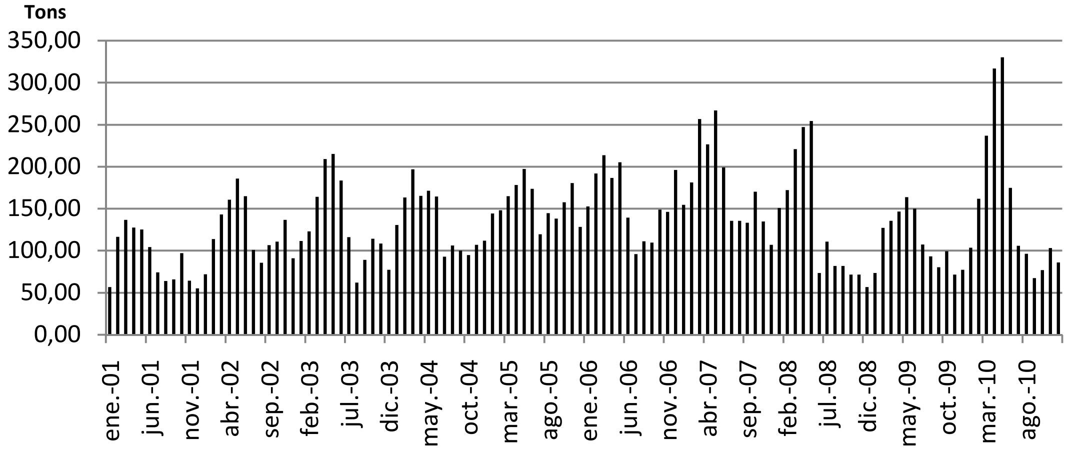


Figure 4.1. Monthly landings of *Eledone cirrhosa* registered in Galician harbors during the period 2001-2010 (data source: www.pescadegalicia.com). The evolution of annual landings shows an increasing trend of catches year by year and a clear annual cyclical pattern, closely linked to the reproductive cycle of the species.

Ecological niche can be thought to conform a n-dimensional space where each dimension represents one environmental variable or resource in which species are distributed according to their particular requirements (Hutchinson, 1957). Marine species-specific habitats, where they can feed, grow, mature and spawn to sustain their populations, are commonly known as Essential Fish Habitat (EFH) as defined by the EC Habitats Directive (Council Directive 92/43/EEC of 21 May 1992 on the Conservation of Natural Habitats and of Wild Fauna and Flora). Characterization of EFH is crucial to improve our knowledge on the relationship among biotic and abiotic factors with life cycle of the species, and a fisheries sustainable management. Generalized Additive Models

(GAMs), a form of General Linear Models (GLM), constitute the most common and well developed method for modelling fish habitats (Valavanis *et al.*, 2008a), and provides the possibility to model different environmental scenarios, including non-linear responses of biological variables to environmental forcing variables. On the other hand, they allow to define the optimal areas of potential distribution for the particular stages of the species life cycle, e.g. areas of larval development and recruits and adults grounds (Sánchez *et al.*, 2008) and seem to represent a good trade-off between model complexity and performance for a diverse set of species (Meynard and Quinn, 2007).

The aim of this paper is to describe migratory behaviour of *E. cirrhosa*, by defining habitat use during three particular stages of its reproductive period, according to catch data registered in two survey series performed in summer-autumn in NW Iberian waters.

Material and methods

Study area

The study area comprised the northwest coast of the Iberian Peninsula, from the Ría of Ribadeo to the Miño river estuary, on the border between Spain and Portugal. For descriptive purposes, and according to main coast orientation, the studied area was divided in three regions: Northern area (N) from Ría of Ribadeo to cape Ortegal, north-western area (NW) from cape Ortegal to cape Finisterre and western area (W) from cape Finisterre to Miño river estuary (Figure 4.2).

Datasets

Capture data from two series of surveys, comprising a total of 276 trawls with a depth range from 50 to 500 m and carried out during summer-autumn in Galician waters, were considered. Dates and geographical area are summarised in Table 4.1. Surveys were performed in the research vessels *Cornide de Saavedra* (*G* surveys) and *Emma Bardán* (*C* surveys). According to characteristics of the boats and fishing gear used (Table 4.2) capture data from both surveys series were considered as comparable.

A preliminary statistical analysis was performed by comparing data between surveys, in order to check whether there are significant differences (at 95%) in abundance (individuals per mile), biomass (grams per mile) and average weight of individuals (g) through depth strata. Since the assumptions of normality and homoscedasticity were not met, multiple comparisons among zones and strata were tested using non-parametric Kruskal–Wallis test and Mann-Whitney for paired comparisons. Bonferroni approach was used to adjust alpha (α_t), in order to control Type I error across tests (Zar, 1999).

According to the season when fishing were performed, and based on reproductive dynamics of *E. cirrhosa*, surveys represent three different stages of the life cycle of the species: reproductive season (G3 survey), post-spawning season (G1 and G2 surveys) and recruitment season (C surveys), which allows a phenological approach to life cycle of the species.

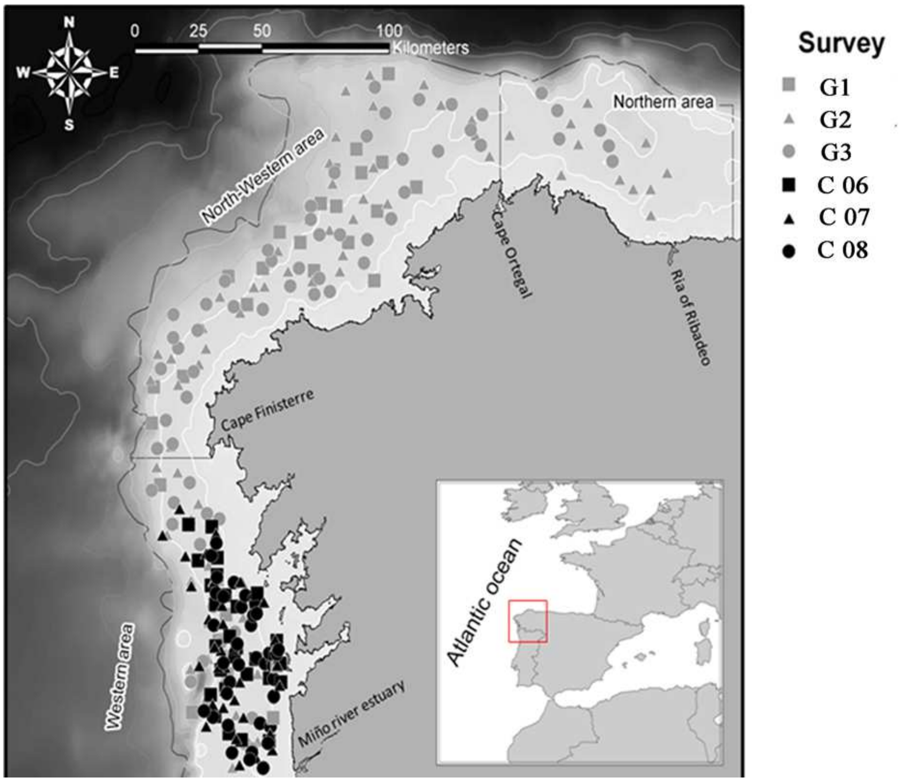


Figure 4.2. Data from three series of surveys have been considered to infer spatial-temporal patterns of *Eledone cirrhosa* in North-western Iberian. The map shows hauls distribution by survey through sampling areas. G series surveys (1974-1976) are drawn in grey and C series surveys (2006-2008) are drawn in black.

Table 4.1. Data from a total of six surveys, corresponding to two series, were considered in order to infer temporal and spatial variations in distribution and abundance of the octopus *Eledone cirrhosa* in north-western Iberian waters. The table shows a summary of the surveys, with information about dates and zones where capture data were obtained.

Survey Series	Survey	Date	Number of hauls	Sampled Zone
Galicia (G)	G1	September 1974	27	From Bares Cape to Miño estuary
	G2	August-September 1975	67	From Ría de Ribadeo to Miño estuary
	G3	June-July 1976	68	From Ría de Ribadeo to Miño estuary
Cigala (C)	C 06	October-November 2006	29	From Finisterre cape to Miño estuary
	C 07	October-November 2007	42	From Finisterre cape to Miño estuary
	C 08	October-November 2006	43	From Finisterre cape to Miño estuary

Table 4.2. Capture data were obtained from two series of surveys carried out in two different boats. Table shows a description of the vessels and fishing gear used. According to their characteristics, data obtained in all six surveys series were considered as comparable.

		Cornide de Saavedra	**Emma Bardán**
Vessel	LOA	58m	29m
	Horsepower	2x625 C.V.	900 C.V.
Fishing gear	leadline lenght	53 m	52 m
	Sweepline lenght	130 m	160 m
	meshsize	35.5 mm	40mm

Capture data were adapted to a digital database for each survey, adding positional information in UTM 29N (datum WGS84). Datasets were transferred to a GIS, allowing spatio-temporal analysis of data (Pierce *et al.*, 2001). During *Galicia* (*G*) surveys, a set of environmental data acquisition sites was associated, registering salinity and temperature in the water column. Since *E. cirrhosa* is a benthic cephalopod, bottom information was selected and then interpolated, in order to add the corresponding information to the biological dataset. Depth and slope information was obtained from GEBCO 30 arc-second bathymetry grid layer, and transferred to each haul record. A series of grid layers was performed representing the distance to the coast, from vectorial coastline layer, and mean net primary production, generated from HCAF v.4 data (Kaschner *et al.*, 2008). Corresponding information were transferred to each data point.

The same methodology was followed for *C* surveys. Although due to the lack of bottom salinity and temperature associated direct measurements, this information was obtained by interpolating information from oceanographic samplings from World Ocean Database, available in NOAA agency website (http://www.nodc.noaa.gov). Information about data source and pre-treatment can be found in Johnson *et al.* (2009). Time-corresponding available records were selected and included into a point file with the available information of each survey. As in *G* surveys, bottom data were selected for each sampled point with the aim of modelling each variable in the ocean bed. A summary of all environmental variables source can be found in Table 4.3. Fishing points were enriched with time-corresponding environmental data from the created continuous layers, establishing our data matrix.

Modelling

Data for each survey were fitted to a GAM model, a nonparametric analogous to a GLM, suitable for solving the problems of planning and management in large territorial areas (Seoane and Bustamante, 2001), and moreover widely used to determine the distribution of a species based on environmental parameters (Valavanis *et al.*, 2008b).

The use of GAMs provides the possibility to model different environmental scenarios, including non-linear responses of biological variables to environmental forcing ones using smooth functions, and to define the optimal areas of potential distribution for the particular stages of the species life cycle (Sánchez *et al.*, 2008). The advantage of this analytical approach is its flexibility modelling complex relationships between response and predictor variables. A specificity of GAM models, as opposed to simple linear models, is that the response variable is modelled by specifying the underlying error distribution of the model and the link function appropriate to each type of variable which relates the response variable to the predictors. In our case, distribution used for this application of the GAM was a Poisson, which is appropriate for describing random occurrences and count data (Sokal and Rohlf, 1981; Zar, 1999), with a log link relating the abundance (individuals per mile) to the predictor variables. To prevent problems of instability of the models through the phenomenon known as multicollinearity, a preliminary analysis of these variables were made, avoiding to include variables with collinearity higher than 80% (Crec'hriou *et al.*, 2008) (Table 4.4).

Table 4.3. A set of environmental variables was added to each model, in order to consider their influence on the distribution and abundance of *E. cirrhosa* throughout their reproductive season. Table shows a summary of environmental variables collected for each survey and source description.

Variable	**Abreviation**	**Description**
UTM Longitude	UTM_LONG	UTM longitude layer Generated from cartography. Units: meters (UTM 29N ;WGS84)
UTM Latitude	UTM_LAT	UTM latitude layer Generated from cartography. Units: meters (UTM 29N ;WGS84)
Depth (m)	D	GEBCO Bathymetry layer 30 arc-second grid generated by combining quality-controlled ship depth soundings with interpolation between sounding points guided by satellite-derived gravity data.
Slope (degrees)	SL	Slope layer generated from bathymetry.
Sea bottom salinity (PSU)	SBS	G surveys: SBS layer generated by interpolation from oceanographic samplings. C surveys: SBS layer generated by interpolation from NOAA agency website registers.
Sea bottom temperature (degrees Celsius)	SBT	G surveys: SBT layer generated by interpolation from oceanographic samplings. C surveys: SBT layer generated by interpolation from NOAA agency website registers.
Mean Primary productivity (mgC*m-2*day-1)	NPP	Annual mean Net Primary Production layer generated by interpolation of HCAF v.4 data.
Distance to coast (m)	DC	Buffer generated from vectorial coastline layer.

Table 4.4. Autocorrelation matrix between layers of environmental variables included in the distribution models. Asterisks indicate correlations greater than 80%; in those cases variables were not included simultaneously to develop each model.

Layer	DISTCOST	LAT	LONG	PPN	PROF	SBS	SBT	SLOPE
DISTCOST	**1,0000***							
LAT	-0,0137	**1,0000***						
LONG	-0,7304	0,4129	**1,0000***					
PPN	-0,7109	-0,3969	0,1898	**1,0000***				
PROF	-0,8184*	-0,2088	0,5890	0,6335	**1,0000***			
SBS	-0,8796*	-0,1750	0,6088	0,8157*	0,8717*	**1,0000***		
SBT	-0,8926*	-0,1094	0,6325	0,8044*	0,8706*	0,9899*	**1,0000***	
SLOPE	-0,0728	0,2013	0,2050	-0,1199	-0,0477	-0,0920	-0,1045	**1,0000***

Bathymetric distribution of the species in Galician continental shelf is still unknown, although there are no records in the littoral zone. Furthermore, its deepest record is around 1000 m in Hebrides slope (Boyle *et al.*, 1998). According to this and in order to obtain a more accurate model, additional absence data were introduced in the model in both coastal waters and in depths higher than 800 m.

All possible models were fitted by permutation of available environmental variables. The best model was considered the one where all variables remained significant after successive elimination of the variables that were not. This way of selection was chosen because after assessing AIC values, we found that some of the best models did not evaluate any of the variables as significant. As a result we obtained a single model for each survey.

The GAMs models developed were implemented by Marine Geospatial Ecology Tools for ESRI ArcGis v.9.3.

Results

Overall comparisons between general abundances, biomass and average weight of all surveys showed significant differences (Kruskal-Wallis test: H (5, N= 276) $p<0.001$). However, according to the analyses of pooled data from the surveys carried out during the same month (*G1* and *G2* surveys and *C* surveys), we cannot reject the null hypothesis of homogeneity ($p>0.01$). Thus, comparisons between general abundance from *G* surveys showed significant differences between *G3* survey and *G1* and *G2* surveys ($p<\alpha_t$). No differences were found between *G1* and *G2* ($p>\alpha_t$), performed in the same season.

Catches per depth strata (individuals per nautical mile) are shown in Table 4.5. Higher abundances were recorded in intermediate depth (100-200 m strata), with the exceptions of North and West Galician regions in *G1* and *G3* surveys, respectively, in which higher abundances were concentrated at 200-500 m strata. *Eledone* abundance was higher in surveys C than in surveys G. In G series the lowest abundances were registered in the north region (N).

Significant differences in abundance were found between 100-200 m and 200-500 m strata in NW region during *G2* and *G3* ($p<\alpha_t$). Abundance in *C 06* also showed differences between 50-100 m and 100-200 m, and in *C 07* were detected dissimilarities between 100-200 m and 200-500 m strata. Some other of the paired comparisons could not be made, or are meaningless, due to the eventual scarcity of hauls for a given stratum.

Biomass data (g/nautical mile) per strata are shown in Table 4.6. In contrast to the abundance data, higher biomasses were generally recorded at greater depths. Significant differences ($p<\alpha_t$) were found between 50-100 m and 100-200 m strata in *C 06* survey and between 100-200 m and 200-500 m strata in *C 07*, and NW region in *G2*. Significant differences were also found combining abundances per strata from *C* surveys between 50-100 and 100-200 and also between 100-200 and 200-500 m ($p<\alpha_t$). Data showed also differences between biomass in 50-100 m and 100-200m but not in average weight.

Corresponding results were found analysing average weight (g) of the animals per depth strata (Table 4.7). Higher average sizes were generally found at deeper strata. Differences in size were also statistically significant between 50-100 m and 100-200 m strata in *C 06* survey and between 100-200 m and 200-500 m strata in *C 07* and NW region in *G2*.

Obtained models are summarized in Table 4.8. Figure 4.3 illustrates the effect of smoothing function for the variables included in the model developed for *G1* survey. Model-derived abundance maps for each survey are shown in Figure 4.4. Model performed for early summer survey (*G3*) showed higher concentration of individuals near the coast, reaching abundances over 64 individuals per mile. Conversely, models corresponding to late summer indicate a general decline in the abundance, reaching maximum values below 20 individuals per mile in *G2* model and even lower for *G1* model. Moreover, *E. cirrhosa* concentrations seem to move offshore reaching 150 - 200 m depths (*G2* model) and even deeper (500 - 2000 m in *G1* model).

Table 4.5. Average individuals per mile (Mean ±S.D.) of *E. cirrhosa* by depth stratum for G and C surveys. Higher abundances were generally recorded at intermediate depth (100-200 m strata). Abundances in C surveys were much higher than those recorded in G

ABUNDANCE	G1		G2			G3			C 06	C 07	C 08
Regions/Strata	Silleiro	Vilano	Silleiro	Vilano	Bares	Silleiro	Vilano	Bares	Silleiro	Silleiro	Silleiro
50-100	0,4 ± 0,4	0,74 ± 0	0,38 ± 0	-	-	-	-	-	0,92 ± 0,23	17,68 ± 4,31	23,51 ± 5,84
100-200	1 ± 0,58	1,07 ± 0,44	1,68 ± 0,49	0,79 ± 0,19	-	1,78 ± 0,57	6,13 ± 0,83	0,24 ± 0,11	24,58 ± 3,18	20,81 ± 2,87	34,49 ± 6,46
200-500	1,62 ± 0,97	0,36 ± 0,25	0,52 ± 0,19	0,11 ± 0,06	-	0,32 ± 0,19	3,3 ± 0,88	0,32 ± 0	-	1,67 ± 1,26	13,54 ± 0

Table 4.6. Average biomass in grams per mile (Mean±S.D.) of *E. cirrhosa* by depth strata for G and C surveys. Biomass was higher in C surveys (Oct-Nov), due to increasing abundance of new recruits.

BIOMASS	G1		G2			G3			C 06	C 07	C 08
Regions/Strata	Silleiro	Vilano	Silleiro	Vilano	Bares	Silleiro	Vilano	Bares	Silleiro	Silleiro	Silleiro
50-100	68 ± 68	333,33 ± 0	26,92 ± 0	-	-	-	-	-	98,14 ± 22,23	1545,86 ± 370,04	6439,68 ± 2369,01
100-200	235,56 ± 127,97	322,69 ± 146,75	109,62 ± 43,35	181,1 ± 51,75	-	447,7 ± 164,85	1515,95 ± 222,27	62,72 ± 32,03	4816,12 ± 936,09	2442,67 ± 302,7	2540,35 ± 311,78
200-500	708,78 ± 402,33	142,99 ± 104,06	110,62 ± 59,06	30,76 ± 17,37	-	101,04 ± 58,64	1049,63 ± 278,95	48,39 ± 0	-	327,67 ± 210,12	3723,96 ± 0

Table 4.7. Average weight of captured individuals in grams (Mean±S.D.) of *E. cirrhosa* by depth strata for G and C surveys. Larger individuals were registered during G3, performed in early summer.

Average weight	G1		G2			G3			C 06	C 07	C 08
Regions/Strata	Silleiro	Vilano	Silleiro	Vilano	Bares	Silleiro	Vilano	Bares	Silleiro	Silleiro	Silleiro
50-100	85 ± 85	450 ± 0	70 ± 0	-	-	-	-	-	97,75 ± 28,01	111,52 ± 33,02	260,25 ± 52,94
100-200	162,22 ± 82,22	214,35 ± 49,95	50,59 ± 13,26	145,81 ± 31,79	-	148,05 ± 30,33	251,09 ± 15,18	167 ± 93,08	215,98 ± 47,44	135,04 ± 15,11	88,31 ± 8,86
200-500	451,79 ± 23,21	98,05 ± 51,38	127,36 ± 56,3	63,46 ± 33,8	-	156,25 ± 90,36	295,93 ± 25,62	150 ± 0	-	185,09 ± 159,28	275 ± 0

Table 4.8. Summary of Generalized Additive Models (GAMs) fitted to catches of the horned octopus *E. cirrhosa* obtained during two series of trawl surveys conducted in Galician Waters. A single model was performed for each survey. Table shows a summary of environmental variables included in each model. Significance codes: '***'<0.001; '**' < 0.01; '*'< 0.05; '+'<0.1.

Survey	Region	Variables	Deviance explained	N
G1	NW & W Galicia	s(UTM_LAT) + s(NPP)**	47.7%	86
G2	N, NW & W Galicia	s(UTM_LONG) s(UTM_LAT)** s(SL)*	81%	126
G3	N, NW & W Galicia	s(SBT)** s(UTM_LONG)*** s(UTM_LAT)*** s(SL)***	85%	127
C 06	W Galicia	s(SBS)*** s(UTM_LAT)***	84%	88
C 07	W Galicia	s(SBT)** s(UTM_LONG)*** s(UTM_LAT)*** s(SL)***	83.5%	101
C 08	W Galicia	s(SBT)*** s(UTM_LONG)*** s(UTM_LAT)**	82%	102

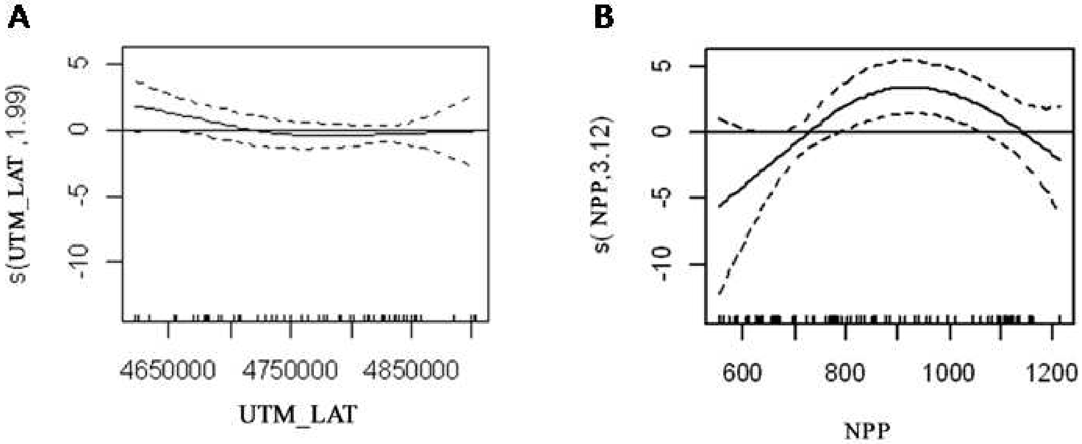


Figure 4.3. Estimated smoothing functions for (A) Latitude (UTM_LAT) and (B) Net Primary Production (NPP) applied to G1 GAM model. The x axis of each plot is labelled with the covariate name, while the y axis is labelled s(cov,edf) where cov is the covariate name, and edf the estimated degrees of freedom of the smooth. The solid line is the predicted value of each dependent variable as a function of the x axis. The standard errors of the estimates are plotted in dashed lines. The small lines along the x axis represent sample points.

Discussion

Landing patterns of *Eledone cirrhosa* from 2000 to 2010 showed a gradual increase and an annual cyclical pattern (Figure 4.1). This marked seasonality is typically associated to the biology of the majority of cephalopods with short life cycles, rapid population turnover and semelparous reproductive strategy (Sánchez and Martín, 1993), and involves particular problems for fisheries management (Giordano *et al.*, 2010).

Particularly for *E. cirrhosa*, it is not expected a bias in commercial landings amount, since it is not a target resource for the trawl fleet and, in consequence, fishery statistics can be interpreted as an indicator of some aspects of population dynamics (e.g. density and abundance).

Commercial landings indicate that the abundance of *E. cirrhosa* decrease substantially through summertime, most likely due to post-spawning mortality as proposed by Casali *et al.* (1998) and post-reproductive migration, as previously suggested by Guerra (1992).

In practice, to use CPUE as an index of abundance, the catchability must be reasonably constant among samples, not coming from very different fishing fleets and/or gears (Petrere Jr. *et al.*, 2010). Thus, our data can be interpreted as abundance indexes. Overall results for *G* surveys, performed in the three studied zones, show lower abundances in the north region than in the centre and south areas. This pattern agrees whit zonal commercial landing statistics (Data not shown).

Differences found between combining abundances and biomasses per strata from *C* surveys, as well as average weight variation, suggest the existence of size segregation in the population, with the new recruits mainly selecting intermediate strata (100-200 m). The existence of size segregation is supported by the facts that the higher biomasses were registered at depth strata, indicating an increase in the average size of the individuals at greater depths, and also by the average size data. A similar situation was found by Sánchez & Martin (1993) in the Catalonian sea. On the other hand, segregation by sex and maturity stage has been pointed out in previous studies (Giordano *et al.*, 2010; Salman *et al.*, 2000; Wurtz *et al.*, 1992).

As expected, higher average individual sizes were registered in summer (*G* surveys) in comparison with autumn (*C* surveys), because the new cohort is still not recruited. In that case, less abundant adults would compose almost all the summer catches. This matched with the decrease of captures registered in commercial landings during summer time (Figure 4.1). On the other hand, higher biomasses were registered in the *C* surveys carried out in autumn (October-November), which correspond to new and abundant recruits.

Due to the lack of data for north and north-western Galician regions during the *C* surveys, our data are inconclusive about the inter-decadal variation of abundance in those areas.

Despite the highly sensitive to environmental conditions, and changes at a range of spatial and temporal scales of cephalopods (Pierce *et al.*, 2008), no significant differences were found between *G1* and *G2* surveys or between *C* surveys, performed at the same dates. This suggested that the pooled data of surveys are useful tools for representing different phenological stages of the species.

According to the developed models, higher abundances are reached at June-July in coastal areas and later, in October-November at higher depths. Moreover, according to the increase of abundance and decrease of overall weight registered in autumn, a decline of adult specimens during summertime and a recruitment of new individuals to the fishing ground is suggested, as was reported in other studies (Giordano *et al.*, 2010; Orsi Relini *et al.*, 2006; Salman *et al.*, 2000).

It is known that, in this area, *E. cirrhosa* disappears in shallow waters. However, despite the inclusion of absence data along coastline, models indicate that the overall bathymetric range of the species includes shallow waters until shoreline (Figure 4.4.A). This result suggests that, from an environmental point of view, potential distribution of the species would include coastal areas. The actual exclusion of the species from this coastal area may be due to biotic factors, not included in the models, such as interspecific competition with species that occupy that niche, as *Octopus vulgaris*. This scenario would be supported since *E. cirrhosa* extends its bathymetric range to shallow coastal waters in northern Europe (Stephen, 1944), beyond the northern limit of *O. vulgaris* distribution (Guerra *et al.*, 2010; Mangold, 1983).

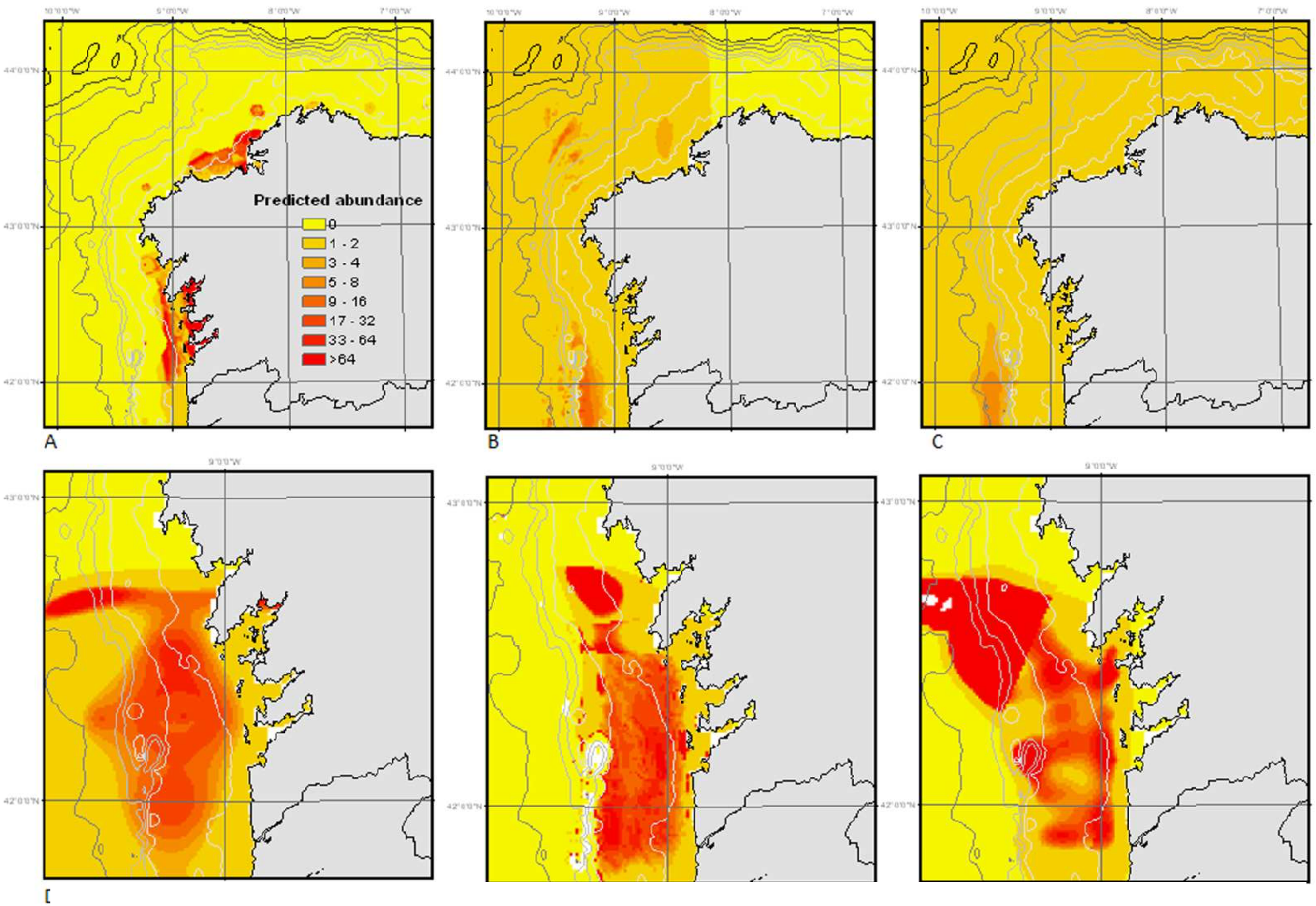


Figure 4.4. Maps of abundance developed by applying GAMs over capture and environmental data and ordered by representing phenological stage. A: G3 (June-July); B:G2 (August-September); C: G1 (September); D:C 06, E: C 07 and F: C 08 (Oct-Nov).

From a phenological perspective, and in view of developed models, higher abundances were predicted near shore in early summer, corresponding to the beginning of spawning season. The models also indicate a displacement of higher abundance areas offshore through the summer, as suggested by Rees (1956). Late summer distribution models showed that the higher abundances were concentrated in deep waters (500-1000 m), which suggest a migration to deeper waters throughout the summer.

Since our surveys did not include strata deeper than 500 m, the outputs of the models should be treated with caution. Thus, from a temporal perspective, predicted high abundance areas at deeper waters cannot be rejected. This migration appeared simultaneously with the post-reproductive decrease of abundance.

Among the environmental variables considered, only the latitude remained significant in all developed models, suggesting that photoperiod, a direct effect of latitude, plays an important role influencing sexual maturation in this species, as suggested by Regueira et al. (2013). The latitudinal effect over the abundance and distribution of *E. cirrhosa* could also be associated to some other related variables, as temperature or net primary production.

Northwestern Iberian coasts constitutes northern limit of the seasonal upwelling event taking place along northeast Atlantic coast, from 12° N to 43°N, where Finisterre cape is placed (Wooster *et al.*, 1976). Upwelling intensity in this area is highly influenced by coastline shape, bathymetry and direction and intensity of dominant winds (Sordo *et al.*, 1999). Since seasonal upwelling has been suggested to influence life-cycle of other cephalopod species, such as *Octopus vulgaris* (Otero *et al.*, 2009; Otero *et al.*, 2008; Rocha *et al.*, 1999), further studies must be performed to analyze possible relationships between *E. cirrhosa* lifecycle and upwelling seasonal event.

Although it is not likely that females perform a parental care, as other octopodidae (Hanlon and Messenger, 1996), this behaviour must be investigated in *E. cirrhosa*.

Acknowledgements

This research was supported by the project "Impacto do ambiente sobre o polbo *Eledone cirrhosa* no sistema de afloramento costeiro das augas de Galicia" (INCITE08PXIB402074PR), granted by the Xunta de Galicia. We thank Secretaría General de Pesca (MAAMA) by providing access to raw data of C surveys. Marcos Regueira was supported by a FCT grant from Portugal.

References

Belcari, P., Tserpes, G., González, M., Lefkaditou, E., B., M., Piccinetti, G. M., Souplet, A., 2002. Distribution and abundance of *Eledone cirrhosa* (Lamarck, 1798) and *E.moschata* (Lamarck, 1798) (Cephalopoda: Octopoda) in the Mediterranean Sea. Sci. Mar. 66, 143-155.

Boyle, P. R., 1983. *Eledone cirrhosa*. in Cephalopod life cycles. Academic Press. 365-386 pp.

Boyle, P. R., 1986. A descriptive ecology of *Eledone cirrhosa* (Mollusca: Cephalopoda) in Scottish waters. J. Mar. Biol. Ass. U. K. 66, 855-865.

Boyle, P. R., Mangold, K., Ngoile, M. A., 1988. Biological variation in *Eledone cirrhosa* (Cephalopoda: Octopoda): Simultaneous comparison of North Sea and Mediterranean populations. Malacologia. 29, 77-87.

Boyle, P. R., Collins, M. A., Williamson, G. R., 1998. The cephalopod by-catch of deep water trawling on the Hebrides slope. J. Mar. Biol. Ass. U. K. 78, 1023-1026.

Casali, G., Piccinetti, M., Soro, S., 1998. Distribuzione di cefalopodi in Alto e Medio Adriatico. Biol. Mar. Medit. 5, 307-318.

Crec'hriou, R., Bonhomme, P., Criquet, G., Cadiou, G., Lenfant, P., Bernard, G., Roussel, E., Diréach, L. L., Planes, S., 2008. Spatial patterns and GIS habitat modelling of fish in two French Mediterranean coastal areas. Hydrobiologia. 612, 135-153.

Giordano, D., Busalacchi, B., Bottari, T., Perdichizzi, F., Profeta, A., Perdichizzi, A., Pirrera, L., Modica, L., Rinelli, P., 2010. Population dynamics and distribution of *Eledone cirrhosa* (Lamarck, 1798) in the Southern Tyrrhenian Sea (Central Mediterranean). Cah. Biol. Mar. 51, 213-227.

Guerra, A., 1992. Mollusca, Cephalopoda. Fauna Ibérica. Museo Nacional de Ciencias Naturales. CSIC. Madrid. 1, 327 pp.

Guerra, A., Roura, A., Gonzalez, A. F., Pascual, S., Cherel, Y., Perez-Losada, M., 2010. Morphological and genetic evidence that *Octopus vulgaris* Cuvier, 1797 inhabits Amsterdam and Saint Paul Islands (southern Indian Ocean). ICES J. Mar. Sci. 67, 1401-1407.

Hanlon, R. T., Messenger, J. B., 1996. Cephalopod Behaviour. Cambridge University Press. 232 pp.

Hutchinson, G.E. (1957). Concluding remarks. Cold Spring Harbor Symposia on Quantitative Biology 22 (2): 415–427.

Johnson, D. R., Boyer, T. P., Garcia, H. E., Locarnini, R. A., Baranova, O. K., Zweng, M. M., 2009. World Ocean Database 2009 Documentation. Edited by Sydney Levitus. NODC Internal Report 20, Silver Spring, MD, 175 pp. Available at http://www.nodc.noaa.gov/OC5/WOD09/pr_wod09.html.

Kaschner, K., J.S. Ready, E. Agbayani, J. Rius, K. Kesner-Reyes, P.D. Eastwood, A.B. South, S.O. Kullander, T. Rees, C.H. Close, R. Watson, D. Pauly and R. Froese 2008. AquaMaps Environmental Dataset: Half-Degree Cells Authority File (HCAF). World Wide Web electronic publication.

Lefkaditou, E., Souplet, A., Peristeraki, N., Gonzalez, M., Kavadas, S., Vidoris, P., Cuccu, D., Papaconstantinou, C., 2000. Preliminary investigation of factors affecting the spatial distribution and abundance of *Eledone cirrhosa* (cephalopoda: octopoda) in the Mediterranean Sea. In: Demersal resources in the Mediterranean Éd. Ifremer, Actes Colloq. 26, 53-63.

Mangold-Wirz, K., 1963. Biologie des Céphalopodes bentiques et nectoniques de la Mer Catalane. Vie Milieu Ser. C Biol.. 285 pp.

Mangold, K., 1983. Food, feeding and growth in cephalopods. Mem. Natl. Mus. Victoria. 44, 81-93.

Mangold, K., Boletzky, S. v., Frösch, D., 1971. Reproductive biology and embryonic development of *Eledone cirrhosa* (Cephalopoda: Octopoda). Mar. Biol. 8, 109-117.

Meynard, C. N., Quinn, J. F., 2007. Predicting species distributions: a critical comparison of the most common statistical models using artificial species. J. Biogeogr. 34, 1455-1469.

Orsi Relini, L., Mannini, A., Fiorentino, F., Palandri, G., Relini, G., 2006. Biology and fishery of *Eledone cirrhosa* in the Ligurian Sea. Fish. Res. 78, 72-88.

Otero, J., Alvarez-Salgado, X. A., González, A. F., Miranda, A., Groom, S. B., Cabanas, J. M., Casas, G., Wheatley, B., Guerra, A., 2008. Bottom-up control of common octopus *Octopus vulgaris* in the Galician upwelling system, northeast Atlantic Ocean. Mar. Ecol. Prog. Ser. 362, 181-192.

Otero, J., Alvarez-Salgado, X. A., González, A. F., Gilcoto, M., Guerra, A., 2009. High-frequency coastal upwelling events influence *Octopus vulgaris* larval dynamics on the NW Iberian shelf. Mar. Ecol. Prog. Ser. 386, 123-132.

Pertierra J.P., P. Sánchez, 2005. Distribution of four cephalopoda species along the catalan coast (NW mediterranean) using gis techniques. Phuket Mar. Biol. Cent. Res. Bull. 66, 283-289.

Petrere Jr., M., Giacomini, H., De Marco Jr., P., 2010. Catch-per-unit-effort: which estimator is best?. Braz. J. Biol. 70, 483-491.

Pierce, G. J., Wang, J., Zheng, X., Bellido, J. M., Boyle, P., Denis, V., Robin, J. P., 2001. A Cephalopod fishery GIS for the Northeast Atlantic: development and application. INT. J. Geogr. Inf. Sci. 15, 763-784.

Pierce, G. J., Valavanis, V. D., Guerra, A., Jereb, P., Orsi-Relini, L., Bellido, J. M., Katara, I., Piatkowski, U., Pereira, J., Balguerias, E., Sobrino, I., Lefkaditou, E., Wang, J., Santurtun, M., Boyle, P. R., Hastie, L.C., MacLeod, C. D., Smith, J. M., Viana, M., González, A. F., Zuur, A. F., 2008. A review of cephalopod-environment interactions in European Seas. Hydrobiologia. 612, 49-70.

Rees, W. J., 1956. Notes on the European species of Eledone with especial reference to eggs and larvae. Bull.br.Mus.nat.Hist.Zool. 3, 283-291.

Regueira, M., González, A. F., Guerra, Á., Soares, A., 2013. Reproductive traits of horned octopus *Eledone cirrhosa* in Atlantic Iberian waters. J. Mar. Biol. Ass. U. K., available on CJO2013. DOI:10.1017/S0025315413000118.

Rocha, F., Guerra, A., Prego, R., Piatkowski, U., 1999. Cephalopod paralarvae and upwelling conditions off Galician waters (NW Spain). J. Plankton Res. 21, 21-33.

Russel, E. S., 1921. Report on the cephalopods collected by the research steamer “Goldseeker”, during the years 1903-1908. Sci. Invest. 3 45.

Salman, A., Katagan, T., Gücü, A. C., 2000. The Distribution and Fishing of two Mediterranean *Eledone* spp. (Octopoda:cephalopoda) in the Aegean Sea. Turk. J.Zool. 24, 165-171.

Sánchez, P., Martín, P., 1993. Population dinamics of the exploited cephalopod species of the catalan sea (NW Mediterranean). Sci. Mar. 57, 153-159.

Sánchez, P., Demestre, M., Recasens, L., Maynou, F., Martín, P., 2008. Combining GIS and GAMs to identify potential habitats of squid *Loligo vulgaris* in the Northwestern Mediterranean. Hydrobiologia. 612, 91-98.

Seoane, J., Bustamante, J., 2001. Modelos predictivos para la distribución de especies: una revisión de sus limitaciones. Ecología. 15, 9-21.

Sokal, R. R., Rohlf, F. J., 1981. Biometry: The principles and practice of statistics in biological research. 2nd ed.Freeman: San Francisco. 859 pp.

Sordo, I., Triñanes, J. A., Tobar, A., Arias, J., 1999. Estructuras oceánicas en la costa oeste de la Península Ibérica a finales de la estación de afloramiento. Teledetección: avances y aplicaciones. Abstracts del VIII Congreso Nacional de Teledetección. Albacete. España. 297-300.

Stephen, A. C., 1944. The Cephalopoda of Scottish and adjacent waters. Trans. R. Soc. Edimburg. 61, 247-270.

Valavanis, V., Pierce, G., Zuur, A., Palialexis, A., Saveliev, A., Katara, I., Wang, J., 2008. Modelling of essential fish habitat based on remote sensing, spatial analysis and GIS. Hydrobiologia. 612, 5-20.

Wooster, W. S., Bakun, A., Mclain, D. R., 1976. The seasonal upwelling cycle along the Eastern boundary of the North Atlantic. J. Mar. Res. 34, 131-141.

Wurtz, M., Matricardi, G., Belcari, P., 1992. Distribution and abundance of the octopus *Eledone cirrhosa* in the Tyrrhenian Sea, Central Mediterranean. Fish. Res. 13, 53-66.

Zar, J. H., 1999. Biostatistical Analysis, Fourth Edition. Prentice Hall. USA. 663 pp.

CHAPTER 5. Diet and feeding

This chapter is published as M. Regueira, Á.Guerra, C.M. Fernández-Jardón and Á.F. González, 2017. Diet of the horned octopus Eledone cirrhosa *in Atlantic Iberian waters: ontogenetic and environmental factors affecting prey ingestion. Hydrobiologia (2017). 785:159–171.*

Diet of the horned octopus *Eledone cirrhosa* in Atlantic Iberian waters: ontogenetic and environmental factors affecting prey ingestion

Abstract

Growing concern over sustainability of some fisheries has led to a shift in the focus of fisheries management, from a single-target species toward an ecosystem-based management. This process involves identification a suite of indicators, among which ontogenetic and environmental factors affecting prey ingestion should be considered. The present study combines morphological and molecular analysis of stomach contents and Multinomial Logistic Regression (MLR) to understand the dietary regime and factors affecting it in *Eledone cirrhosa* inhabiting Atlantic Iberian waters. Specimens were collected monthly from commercial bottom trawl multispecies fisheries between February 2009 and February 2011 in three fishing grounds (North Galicia, West Galicia and North Portugal). Based on stomach analysis, *E. cirrhosa* ingested mainly crustaceans, followed by teleost fish, echinoderms, molluscs and polychaetes. Molecular analysis of 14 stomach contents confirmed the visual identification of prey items as well as cannibalistic events. Statistical tests found that sex and body weight did not significantly affect prey selection, while season, fishing ground and maturity stage of the animals had a significantly effect on the probability of selecting a particular prey. The ecological implications of MLR modelling results are discussed.

Keywords: Cephalopods, *Eledone cirrhosa*, Diet, Trophic ecology, Atlantic Iberian waters, Multinomial Logistic Regression.

Introduction

Fisheries management to date has often been ineffective, since it has focused on maximizing the catch of a single target species while ignoring habitat, predators, and prey of the target species as well as other ecosystem components and interactions (Pikitch et al, 2004). Therefore, in order to maintain ecosystem quality and sustaining associated benefits, the ecosystem-based fishery management (EBFM) has been proposed as a holistic approach to a more suitable management of ecosystems. Among the ecological interactions in the EBFM approach, trophic level balance should be considered (Busch et al., 2003, Gislason et al., 2000). Moreover, in that ecological approach the influence of environmental parameters in the components and/or processes within the ecosystem should be also considered.

The moderate-sized incirrate octopod *Eledone cirrhosa* (Lamarck, 1798) inhabits continental shelf and slope ecosystem of the north-western Atlantic and the Mediterranean Sea (Belcari et al., 2015). This species is mainly caught in a multispecies trawling fishery targeting mainly hake, megrim, Norway lobster and monkfish. The fishing activity is carried out between 200 and 800 m depth. Landings of *E. cirrhosa* reached an annual average of 1,634 t in the last ten years in NW Iberian Peninsula (pescadegalicia.com) and show a strong seasonality, in accordance with the reproductive cycle of the species, which reproductive event takes place mainly in spring-summer, with a peak in May-June (Regueira et al. 2013). Thus, landings decrease as breeding season progresses (pescadegalicia.com), mostly due to post-reproductive dead of adults. *E. cirrhosa* is mostly harvested for human consumption, although it can be discarded in some cases.

North-west Iberian Peninsula is located on the north-eastern boundary of the North Atlantic subtropical gyre, where a seasonal upwelling event takes place during spring–summer months (Wooster et al., 1976; Bakun and Nelson, 1991). This atmospheric-oceanographic event is more frequent and intense along the western coast than in the northern one and, in consequence, the area studied in the present paper represents a gradient with respect to prevailing oceanographic conditions, which consequently affect local productivity (Álvarez et al., 2010), and therefore prey availability.

Until present, NW Iberian multispecies trawling fishery has been managed focusing on maximizing the catches of hake, which is the most commercial valuable species. Nevertheless, assessing this resource to obtain its Maximum Sustainable Yield (MYS) is being a challenge still not suitably achieved (ICES WGHMM, 2013). That negative results have also occurred with other European marine resources. For that reason common fisheries policy (CFP) recently warned to undertake a fishing assessment management centered in the ecosystem instead of in a single target species (http: //ec.europa.eu/fisheries/cfp/index_en.htm). We echoed this warning and, in consequence, we tackled studying the spatial and temporal variability of the dietary regime of *E. cirrhosa* as well as the effect of some ontogenetic and environmental factors affecting such variability. This approach has considered two aspects. First, as important ecosystem components, cephalopod populations and their fluctuations directly influence population dynamics of both higher predators and their own prey (Pierce et al., 2008). Second, that our knowledge on biotic communities, trophic web and the influence of environmental conditions in the continental shelf and slope ecosystem of the NW Iberian Peninsula is very scarce and geographically fragmented (Serrano et al., 2011), which connote that the application of an EBFM in the marine realm is far from being applied.

Additionally, the present study would be of interest for aquaculture, which is increasing in the last decade (FAO, 2014). Despite that cephalopods have high rates of growth and food conversion, which for aquaculture translates into short culture cycles, and in addition they have high ratios of production to biomass and high cost-effectiveness, culturing is still in its infancy (Iglesias et al. 2014). Biological traits and captive experiments carried out to date with *E. cirrhosa* (Boyle, 1981; Boyle & Knobloch, 1984; Mangold et al., 1971) showed that this octopus species satisfies all requirements to be considered a potential resource for culture with experimental and/or commercial purposes. Nevertheless, octopod nutrition is one of the biggest challenges for developing culture techniques, since it constitutes a key factor for proper growth and survival under captive conditions (Navarro et al., 2014). In consequence, the results obtained in the present paper could be considered a relevant first step to explore *E. cirrhosa* as a suitable species for culture.

There are some problems when visually characterising the diet of *E. cirrhosa*, mainly because hard parts of their prey, that are usually necessary for identification, are torn into small pieces and often rejected. It has also been observed that rapid digestion means that many specimens have little or no food in their stomachs (Boyle & Rodhouse, 2005). Another major limitation is that stomach contents represent the last feeding events with no indication of long-term dietary habits. Despite these problems, there is a considerable amount of information on the trophic relationships of cephalopods that has been collected using conventional visual analysis of the stomach contents of specimens from fisheries, laboratory studies and analyses of prey remains around middens in the case of some coastal octopods (Mather, 1991; Nixon, 1987). Among the other methodologies used in cephalopod dietary studies (e.g. stable isotopes analysis, lipid signature or DNA sequencing), only serological analysis has been used to identify prey in *E. cirrhosa*. Using this technique, Boyle et al. (1986) demonstrated that *E. cirrhrosa* from Scottish waters preys on several crustacean species. However, the method is too expensive to be used to identify all prey (Boyle & Rodhouse, 2005).

Available dietary information concerning wild *E. cirrhosa* point out that this species mainly preys on decapod crustaceans, mostly alpheids and brachyurans, although molluscs and the eggs of other cephalopods have also been reported as prey to a lesser extent in the Mediterranean (Auteri et al., 1988; Ezzeddine et al., 2012; Moriyasu, 1984; Sánchez, 1981). Cannibalistic behaviour has also been reported (Guerra, 1992; Moriyasu, 1981). No detailed studies on the diet of this species in the Atlantic exist, although studies with a more general approach support the predominance of crustaceans (Boyle, 1986).

Moving on to other issue, advances in statistical modelling have facilitated increasingly sophisticated approaches in ecological surveys that study the effects of multiple explanatory variables and their interactions on a dependent variable. These analyses are becoming more frequent for modelling processes in cephalopods with diverse objectives, including foraging behaviour (Pierce et al., 2008). Thus, Leite et al. (2009) used Multinomial Logistic Regression (MLR) to assess relationships between individual (*Octopus insularis* size) and environmental (e.g. depth, substrate) variables and the occurrence of the three main foraging behaviours and also to assess the effects of the previously mentioned variables in addition to the swimming and moving behaviour on the four main body patterns. As far as we know, this is the only case in which this technique has been used in cephalopod dietary studies.

The aim of this paper is to provide a comprehensive view of the role of *E. cirrhosa* in the marine trophic web through the identification of its dietary regime in Atlantic Iberian waters, based on both visual and genetic identification of the gut contents from wild caught animals as well as checking possible effects of some ontogenetic and environmental factors on the dietary composition using MLR.

Materials and Methods

A total of 2335 specimens of *E. cirrhosa* were obtained from commercial landings in Atlantic Iberian waters. Samples were acquired between February 2009 and February 2011 in Burela’s port (northern Galician fishing ground; NG), Ribeira or Bueu ports, (west Galicia fishing grounds; WG), and Aveiro (western Portuguese fishing ground; WP) (Figure 5.1). All specimens were sexed, and body weight (BW) was measured to the nearest g. The macroscopic maturity scale of Inejih (2000) was adapted to assign a specific maturity stage to each individual. For males, this scale was as follows: I: immature, II: maturing, III: pre-spawning, with some spermatophore in Needham’s sac, and IV: mature, fully developed spermatophore; and for females: I: immature, II: maturing, III: pre-spawning, IV: mature and V: post-spawning. Four size classes were considered (S1: 0-250 g, S2: 251-500 g; S3: 501-750 g, and S4: >750 g) for Emptiness Index (EMI) comparisons. On the basis of the atmospheric-oceanic seasonality described in the area (Bashmachnikov et al., 2015) and in order to determine whether there were any difference in diet between seasons, samples weregrouped into two intervals: “warm season” (April to September) and “cold season” (October to March).

Stomachs were preserved in 70% ethanol until further examination in the laboratory. Stomachs and their contents were weighted separately to the nearest 0.01 g. Stomach content was filtered through a 300 μm mesh to remove silt and organic detritus that would hinder identification of the remaining contents. Hard structures, namely otoliths, fish vertebrae and jaws, pedunculated eyes, traces of chelae and other hard pieces of the body of crustaceans, echinoderm ossicles, pieces of shells of molluscs or traces of cephalopod beaks and radula, were identified to the lowest possible taxon by means of available bibliography (Bouvier, 1940; González-Gurriarán & Mendez, 1986; Guerra, 1992; Perrier, 1954; Tuset et al., 2008; Watt et al., 1997; Zariquiey, 1968). All remains were counted, and minimum number of items was estimated for each stomach. In the case of no quantifiable material present, minimum number of items was recorded as one.

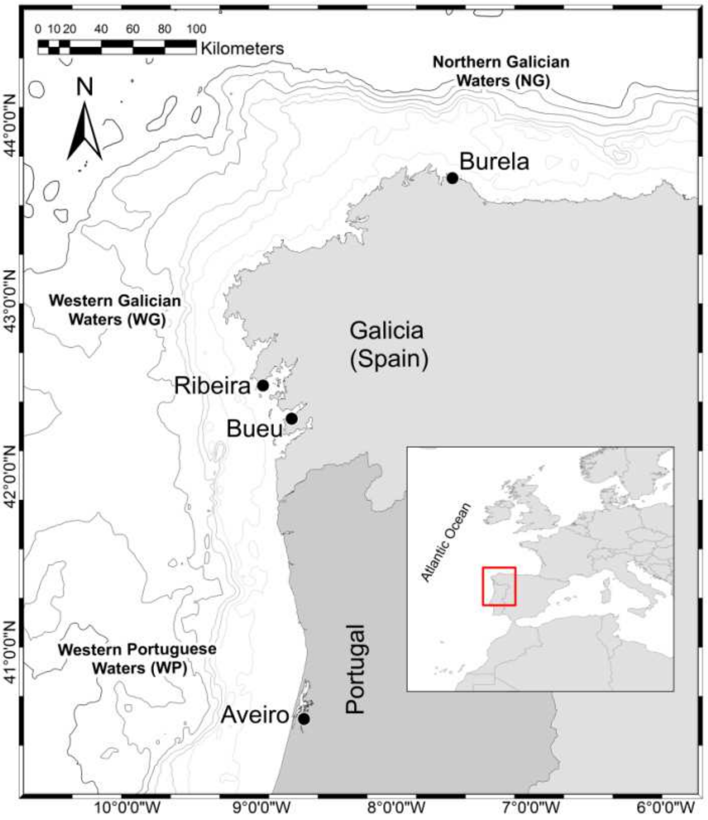


Figure 5.1. Map showing the location of the fishing ports where samples for this study were obtained.

A subset of 21 samples of no identifiable tissues was randomly caught from the stomachs of 20 individuals. Tissue samples were labelled, fixed with 95% ethanol and stored for genetic analysis. Genomic DNA was extracted from each tissue sub-sample by homogenisation and digestion using NucleoSpin® tissue extraction kit, following manufacturer's instructions. Absorbance ratio at 260/280 nm was used to assess the purity of the extracted DNA, with values from 1.8 to 2 representing highly purified DNA (Gallagher & Desjardins, 2006). Cytochrome c oxidase subunit I (COI) was amplified using HCO and LCO universal primers (Folmer et al., 1994). Cycling conditions were as follows: initial denaturation at 94ºC 1 min, followed by 39 cycles of denaturation at 94ºC for 15 seconds, annealing for 30 seconds at 48 Cº, extension at 72ºC for 45 seconds and final elongation for 7 minutes at 72 ºC. Reaction mix was composed by 1,5 µl MgCl$_2$, 2,50 Buffer 10x, 0,2 µl dNTP (10mM), 0,50 µL of each primer (10 µM), 0.13 µl Roche Applied Science Taq DNA polymerase (5 U/µl), 1 µl of DNA and distillate water until 25 µl. 2 µl of each PCR product were checked on 1.5% agarose gels. Those that present a clear band of expected size were clean using USB® ExoSAP-IT® PCR Product Cleanup following manufacturer protocol and sequenced by Sanger sequencing. Obtained DNA sequences were managed (aligned) with MEGA 6 software (Tamura et al., 2013) and compared to sequences in GenBank using BLAST algorithm. Genetically identified preys were added to the main database for further diet analyses.

In order to assess feeding habits, four indexes were calculated: (i) Index of Occurrence (%F), which correspond to the percentage of full stomachs containing a particular prey category; (ii) Percentage by Number (%N), the percentage of each category of prey compared to the total number of prey consumed; (iii) Percentage by weight (%W), total weight of each food category expressed as the percentage of the total weight of all stomach contents. When more than one type of prey was found in a single stomach, and because it was impossible to discriminate one from each other, the total weight of the stomach content was divided by the total number of prey items (Castro & Guerra, 1990); and (iv) Emptiness Index (EMI), the number of empty stomachs compared with the total number of stomachs, expressed as a percentage. Comparison between EMI in each size class was carried out using a Chi square test.

The relative importance of each item group was estimated using two different indices: (i) Feeding Coefficient (Q= %N * %W)(Hureau, 1970); considering main prey for Q>200, secondary for 20<Q<200 and occasional for Q<20 and (ii) Index of Relative Importance (IRI = (%N + %W) * %F)(Pinkas et al., 1971). Pearson’s correlation between both indexes was estimated.

Multinomial logistic regression (MLR) was used to analyse *E. cirrhosa* dietary regime. MLR is useful to predict the probability of category membership on a multinomial dependent variable based on multiple independent variables (Starkweather & Moske, 2011). In the present case, the dependent variable was prey item, categorized into the five main zoological groups consumed. The variables “Sex”, “BW”, “Maturity stage”, “Season” and “Fishing ground”, as well as all possible interactions between them, were considered as explanatory variables. Preliminary assessment of multicollinearity was performed using the condition index of the tetrachoric matrix (Belsley, 2004). Our sample fit the requirement of a minimum sample size of 10 cases per independent variable (Schwab, 2002). A forward stepwise procedure was applied to select the variables in the model. To evaluate the global impact of the independent variables on the dependent one, i.e., the effect of introducing the independent variable in the model, a Likelihood Ratio Test (LRT) was used. In LRT, the Chi-square parameter is the difference of Log likelihood-2 between the final and the reduced model, which is made by omitting an effect of the final model. The null hypothesis is that all the parameters of that effect are zero. To evaluate the impact of each category of independent variables on the dependent one the Wald test was used. To avoid multicollinearity the category "Crustacea" was used as reference in the dependent variable. Specifically in MLR, the dependent variable is the logratio, i.e. the logarithm of the ratio between the probability of preying upon a particular prey category and the probability of eating crustaceans, which is the reference group. The relative direction and strength of each relative effect is given by the parameter β and, therefore, Exp(β) expresses the relative probability of preying a particular item. Furthermore, one particular category of each variable (e.g. NG within "Fishing ground") is selected as reference and the values of the parameters in the model (β) of the remaining cathegories (WG and WP) within that variable ("Fishing ground") are referred to the selected one (NG in this case).

As showed in Table 5.1, polychaetes were an uncommon prey, which would generate singularities in the hessian matrix and, consequently, this prey category was ruled out of the model.

The value of log likelihood-2 (deviation) indicates up to what point the model adjusts well to the information (smaller, better adjustment). The Pseudo R-Square of McFadden, Cox and Snell and Nagelkerke were treated as measures of effect size, similar to how R^2 is treated in standard multiple regressions. Furthermore, Pearson and deviance goodness-of-fit tests were used as a comparison between observed and predicted values, under the null hypothesis of the equality of the considered values.

The statistical software SPSS 23.0 was used.

Results

Diet description

Of the 2335 stomachs examined, only 618 contained prey items. In 120 of them, these contents were mainly composed of semi-digested fleshy material, which were not visually identifiable. In the remaining 498 stomachs, 64.1%of them presented only one type of prey, 25.7% presented two types of prey, and 7.83% three different prey items. More than three types of prey items were detected in 2.37% of the stomachs with prey items. The maximum number of prey items recorded in a single stomach was seven, belonging to a mature female of 243 g BW. The mean number and standard deviation of different prey by stomach were 1.91 and 1.07, respectively.

Empty stomachs represented 73.79% of the total. In overall, EMI did not show significant differences between sexes in any fishing ground (χ^2= 0.26, p>0.05). EMI exhibited monthly variability, ranging from 26.47% to 92.1%, with lower values during the cold season in the three fishing grounds. Regarding size classes, EMI values significantly decreased as BW increased (χ^2= 105.9, d.f. =3, p<0.05). Specifically, EMI corresponding to S1, S2, S3 and S4 size classes were 82.61%, 69.32%, 56.05% and 46.67%, respectively.

Table 5.1 shows the prey items visually and genetically identified as well as calculated indexes for each prey category. Of the 21 soft prey tissues molecularly analyzed, only 15 provided successfully identification. Prey items detected by this technique were: 4 decapod crustaceans (*Polybius henslowii*, *Goneplax rhomboides*, *Munida rugosa* and *Liocarcinus holsatus*), the octopod *Eledone cirrhosa* and the chaetognath *Sagitta enflata*. Results of visual inspection were confirmed by molecular analysis in 71.2% of cases. The partial identification obtained by visual analysis was completed in 50% of the samples. In addition, molecular analysis provided new items in 21% of the samples. Molecular analyses rectified the visual identification of a single sample, which was initially classified as *Galathea* sp. and finally was found to be *Munida rugosa*.

Calculated values of different indexes for each prey category are summarized in Table 5.1. The most important prey species by frequency of occurrence (F%) was the snapping shrimp *Alpheus glaber*, present in a 20.88% of the examined stomachs and comprising

	%F	%N	%W	IRI	Q
POLYCHAETA	2.81	2.09	0.66	7.72	1.38
CRUSTACEANS	88.15	70.64	71.46	12526.81	5048.19
Malacostraca	54.62	45.01	43.16	4815.66	1942.59
Amphipoda	0.80	0.60	0.24	0.67	0.15
Decapoda	53.61	44.26	42.69	4661.83	1889.50
Anomura	6.43	4.92	3.45	53.75	16.95
Family: Galatheidae					
Galathea sp.	4.02	3.13	2.06	20.84	6.45
Galathea squamifera	0.20	0.15	0.08	0.05	0.01
Family: Munididae					
Munida rugosa	0.20	0.15	0.35	0.10	0.05
Munida sp.	0.20	0.15	0.06	0.04	0.01
Family: Paguridae					
Anapagurus laevis	0.40	0.30	0.12	0.17	0.04
UnndentifiedAnomura	1.41	1.04	0.78	2.56	0.81
Brachyura	8.23	6.11	6.34	102.52	38.75
Family: Atelecyclidae					
Atelecyclus undecimdentatus	0.20	0.15	0.06	0.04	0.01
Family: Goneplacidae					
Goneplax rhomboides	5.62	4.17	2.96	40.09	12.34
Family: Homolidae					
Paromola cuvieri	0.40	0.30	0.34	0.25	0.10
Family: Portunidae					
Liocarcinus holsatus	0.20	0.15	0.10	0.05	0.01
Polybius henslowii	1.61	1.19	2.84	6.48	3.39
Unidentified Portunidae	0.20	0.15	0.05	0.04	0.01
Caridea					
Family: Alpheidae					
Alpheus glaber	20.88	19.82	11.44	652.81	226.72
Unidentified Decapoda	27.11	20.12	21.46	1127.17	431.78
Unidentified Crustacea	36.14	27.57	23.97	1863.03	660.96
PISCES	19.68	16.84	17.73	680.23	298.52
Chondrichthyes	0.20	0.15	0.13	0.06	0.02
Osteichthyes	19.48	16.69	17.60	667.95	293.79
Argentiniformes					
Argentina sp.	0.20	0.15	0.11	0.05	0.02
Clupeiformes	0.40	0.30	0.35	0.26	0.10
Perciformes	8.43	8.49	3.89	104.42	33.02
Callionymidae	6.02	6.56	2.28	53.25	14.97
Callionymus maculatus	0.20	0.15	0.02	0.03	0.00
Callionymus sp.	5.82	6.41	2.26	50.50	14.50
Gobiidae	4.02	3.13	1.60	19.01	5.02
Pleuronectiformes					
Arnoglosus sp.	0.20	0.15	0.08	0.05	0.01
Scorpaeniformes					
Triglalyra	0.20	0.15	0.12	0.05	0.02
Unidentified Osteichthyes	11.04	8.20	13.06	234.72	107.02
ECHINODERMS	7.23	5.51	2.53	58.15	13.95
Ophiuridae	2.81	2.24	1.08	9.31	2.41
Unidentified Echinodermata	4.42	3.28	1.45	20.90	4.76
MOLLUSCS	6.43	4.92	7.62	80.57	37.48
Cephalopoda	4.42	3.28	5.98	40.92	19.62
Octopoda					
Eledone cirrhosa	1.00	0.75	0.52	1.27	0.39
Teuthida					
Alloteuthis subulata	0.20	0.15	0.14	0.06	0.02
UnidentifiedCephalopoda	3.21	2.38	5.32	24.74	12.68
Gastropoda	0.40	0.45	0.13	0.23	0.06
Unidentified Mollusca	1.81	1.34	1.51	5.15	2.02

Table 5.1. Percentage of occurrence (%F), percentage by number (%N), weight percentage (%W), index of relative importance (IRI) and feeding coefficient (Q) of prey items visually and molecularly identified in the diet of *Eledone cirrhosa.*

19.82% of the identified items. This prey was follow by the bony fish *Callionymus sp.* (6.02%) and the crab *Goneplax rhomboides*(5.62%).

Across prey groups, F% of crustaceans was 88.15%. The F% for crustacean decapods was 53.6%. Teleost or bony fish appeared in 19.48% of the examined stomachs, and the most preyed upon order was Perciformes, with 8.43%F. Remains of molluscs appeared in 6.43% of the analysed stomachs. Among them, two species of cephalopods were identified: *Eledone cirrhosa* (1%F) and *Alloteuthis subulata* (0.2% F). *E. cirrhosa* remains were found in five specimens (2 from NG and 3 from WG fishing grounds), with weights ranging from 159 to 673 g. Echinoderms were present in 7.23% of cases and, finally, the least abundant prey group was polychaetes, with 2.81%F.

Dietary indexes calculated for each prey category are shown in Table 5.1. Q and IRI indexes showed a strong linear correlation (r^2=0.99).

Dietary regime

The condition index of the tetrachoric matrix was used as an approach to assessing multicollinearity and was found to be 3.60, well below the limit of 10 suggested in the literature (Belsley, 2004). Likelihood ratio test found significant differences between the final and null model (χ^2 = 81.763; 27 d.f; p <0.001). Values for the different pseudo R-Squares were as follows: McFadden = 0.071, Cox and Snell = 0.107, and Nagelkerke= 0.134. Pearson and deviance goodness-of-fit tests did not allowed to refuse the null hypothesis of a proper fitting (p>0.05). Global effects likelihood ratio test (Table 5.2) indicates which variables significantly affect prey selection. According to this test, the probability of preying on a particular zoological group is significantly affected by the "Fishing ground" and the interaction between "Season" and "Maturity stage". The fact that this interaction resulted significant implies the noteworthy effect of both variables separately, although its effect acts in a different way depending on the value taken by the other. On the contrary, "Sex" and "BW" did not influence the diet of *E. cirrhosa* in Atlantic Iberian waters.

Effect	**Fitting model criteria**	**Likelihood ratio contrast**		
	Log likelihood -2 of the reduced model	**Chi-square**	**d.f.**	**Sig.**
Intercept	858,97[a]	0,00	0	-
Season x Maturity	909,55	50,58	21	0,00*
Fishing Ground	891,12	32,14	6	0,00*

Table 5.2. Likelihood Ratio Test (LRT) between the final and the reduced model. ([a]) This reduced model is equivalent to the final model, because omitting the effect does not increase the degrees of freedom; (*) indicates significant effect over probability of *Eledone cirrhosa* prey consumption.

Parameter estimations of the final model are shown in Table 5.3, which shows which concrete categories of each variable affect relative probability of feeding a particular prey category. The intercept parameters indicate how the variable behave in the basic situation, which is defined by the factors designated as reference (in this case “Season” = warm, “Maturity stage”= IV and “Fishing ground” = NG). Hence, the model predicts a significant relative variation in the probability of bony fish consumption with respect to the reference group (crustaceans) depending on the fishing ground, which specifically decreased southwards. Moreover, the model establishes a significant decrease in the relative contribution of bony fish in the diet of individuals in maturity stage II during the warm season. Dietary modelling also suggests that consumption of molluscs with respect to the reference group is lower during the cold season in maturity stages I and II. On the other hand, according to the model, there seems to be no changes in echinoderms consumption with respect to crustaceans. Dietary composition according to significant variables highlighted by the model is shown in Figure 5.2.

Discussion

DNA identification techniques can help identify decomposed tissues in the gut contents of cephalopods (Symondson, 2002). Nevertheless, at present, very few papers have been published on this subject (Deagle et al., 2005; Roura et al., 2012). Despite the limited number of successfully identified prey items by molecular techniques in the present paper,

Prey item group^a		β	Standard error	Wald	d.f.	Sig.	Exp(β)	95% confidence interval for Exp(B)	
								Lower limit	Upper limit
Fish									
	Intercept	-0.893	0.261	11.716	1	0.001*	-	-	-
	Season:Cold * Maturity:I	-0.154	0.374	0.169	1	0.681	0.857	0.412	1.784
	Season:Cold * Maturity:II	0.022	0.317	0.005	1	0.944	1.022	0.549	1.903
	Season:Cold * Maturity:III	-0.581	0.386	2.263	1	0.133	0.559	0.262	1.193
	Season:Cold * Maturity:IV	-0.779	0.481	2.629	1	0.105	0.459	0.179	1.177
	Season:Warm *Maturity:I	-0.634	0.483	1.722	1	0.189	0.531	0.206	1.367
	Season:Warm *Maturity:II	-1.400	0.580	5.826	1	0.016*	0.247	0.079	0.769
	Season:Warm *Maturity:III	-0.336	0.519	0.418	1	0.518	0.715	0.259	1.977
	Season:Warm *Maturity:IV	0^b	-	-	0	-	-	-	-
	Fishing Ground;WP	-3.074	1.020	9.088	1	0.003*	0.046	0.006	0.341
	Fishing Ground;WG	-0,700	0,238	8,674	1	0,003	0,497	0,312	0,791
	Fishing Ground;NG	0^b	-	-	0	-	-	-	-
Echinoderm									
	Intercept	-3.072	0.550	31.145	1	0.000*	-	-	-
	Season:Cold * Maturity:I	0.346	0.651	0.282	1	0.596	1.413	0.394	5.062
	Season:Cold * Maturity:II	0.813	0.583	1.947	1	0.163	2.255	0.720	7.068
	Season:Cold * Maturity:III	0.381	0.647	0.346	1	0.556	1.464	0.412	5.207
	Season:Cold * Maturity:IV	-0.847	1.137	0.555	1	0.456	0.429	0.046	3.982
	Season:Warm *Maturity:I	-19.962	0.000	-	1	-	2.140E-9	2.140E-9	2.140E-9
	Season:Warm *Maturity:II	-1.032	1.134	0.828	1	0.363	0.356	0.039	3.287
	Season:Warm *Maturity:III	-20.129	0.000	-	1	-	1.812E-9	1.812E-9	1.812E-9
	Season:Warm *Maturity:IV	0^b	-	-	0	-	-	-	-
	Fishing Ground;WP	0.509	0.517	0.972	1	0.324	1.664	0.604	4.581
	Fishing Ground;WG	0,298	0,390	0,583	1	0,445	1,347	0,627	2,896
	Fishing Ground;NG	0^b	-	-	0	-	-	-	-
Mollusc									
	Intercept	-1.993	0.380	27.488	1	0.000*	-	-	-
	Season:Cold * Maturity:I	-1.756	0.795	4.882	1	0.027*	0.173	0.036	0.820
	Season:Cold * Maturity:II	-1.215	0.568	4.580	1	0.032*	0.297	0.097	0.903
	Season:Cold * Maturity:III	-0.870	0.571	2.326	1	0.127	0.419	0.137	1.282
	Season:Cold * Maturity:IV	0.241	0.516	0.218	1	0.641	1.272	0.463	3.495
	Season:Warm *Maturity:I	-1.785	1.070	2.785	1	0.095	0.168	0.021	1.365
	Season:Warm *Maturity:II	-1.981	1.065	3.462	1	0.063	0.138	0.017	1.112
	Season:Warm *Maturity:III	-1.754	1.075	2.662	1	0.103	0.173	0.021	1.423
	Season:Warm *Maturity:IV	0^b	-	-	0	-	-	-	-
	Fishing Ground;WP	-0.088	0.664	0.018	1	0.894	0.915	0.249	3.362
	Fishing Ground;WG	0,086	0,394	0,047	1	0,828	1,089	0,504	2,356
	Fishing Ground;NG	0^b	-	-	0	-	-	-	-

Table 5.3. *Eledone cirrhosa* feeding patterns. Final model parameters estimation for Multinomial Logistic Regression (MLR); (^{a}) Reference category was Crustacea; (^{b}) This parameter is 0 because this category is redundant; (*) Indicates signification (Sig<0.05).

it is striking that the majority of prey identified by this technique were decapods, reinforcing the results obtained from visual analyses. On the other hand, it will have to be borne in mind possible misleading results. For instance, the genetically identified chaetognath *Sagitta enflatta* was discarded as prey because it was considered a secondary prey species due to the “Russian dolls” effect, that is, the gut contents of an octopus also included the gut contents of a smaller predator it has ingested, and whose prey was molecularly detected.

Our results show that crustaceans constitute the basis of the diet of *E. cirrhosa* in Atlantic Iberian waters, supporting the general dietary pattern obtained for this species in other studies (Sánchez, 1981; Ezzeddine et al., 2012). This kind of prey was the main prey item present in the diet of *E. cirrhosa* from the Gulf of Lion, with a frequency of >50% in both sexes year round, although the species also feeds upon fish, gastropods, cephalopods, polychaetes, and ophiuroids (Moriyasu, 1981), though at lower frequencies. Accordingly, in confined experiments carried out in the Zoological Laboratory (Aberdeen, Scotland), the species feeds on a wide variety of crustaceans, from lobsters to hermit crabs, including *Carcinus* (Boyle, 1983). However molluscs, when offered, were very rarely eaten in captivity (Boyle, 1983), which agrees with the relative scarcity (6.43%) of this kind of prey found in the present study. The composition of the diet of *E. cirrhosa* described herein also shows clear similarities with its congeneric *E. moschata* from the Adriatic Sea, which preferentially feeds on crustaceans and fish (Sifner & Vrgoc, 2009).

Previous studies on the distribution and abundance of crustaceans in the Galician shelf (González-Gurriarán & Olaso, 1987) have indicated that the most frequent items found in the stomachs of *E. cirrhosa* (*A. glaber* and *G. rhomboides*) actually show low abundances in the wild, with a merely occasional presence in the study area, while other species such as *Munida* spp. and *P. henslowii* exhibit a patchy distribution, being locally abundant. However, relative importance indexes obtained by *P. henslowii* were notable while *Munida* spp. was present in the diet at very low frequencies. Although diet composition is, logically, a compromise between food preference and availability, as well as between the energy obtained and the vulnerability to be detected by a predator during foraging activity, the abovementioned facts suggest that, despite the wide variety of prey available, *E.*

cirrhosa is not an entirely opportunistic hunter but rather a selective generalist, as noted by Anderson (2008) for *O. vulgaris* in the Caribbean Sea.

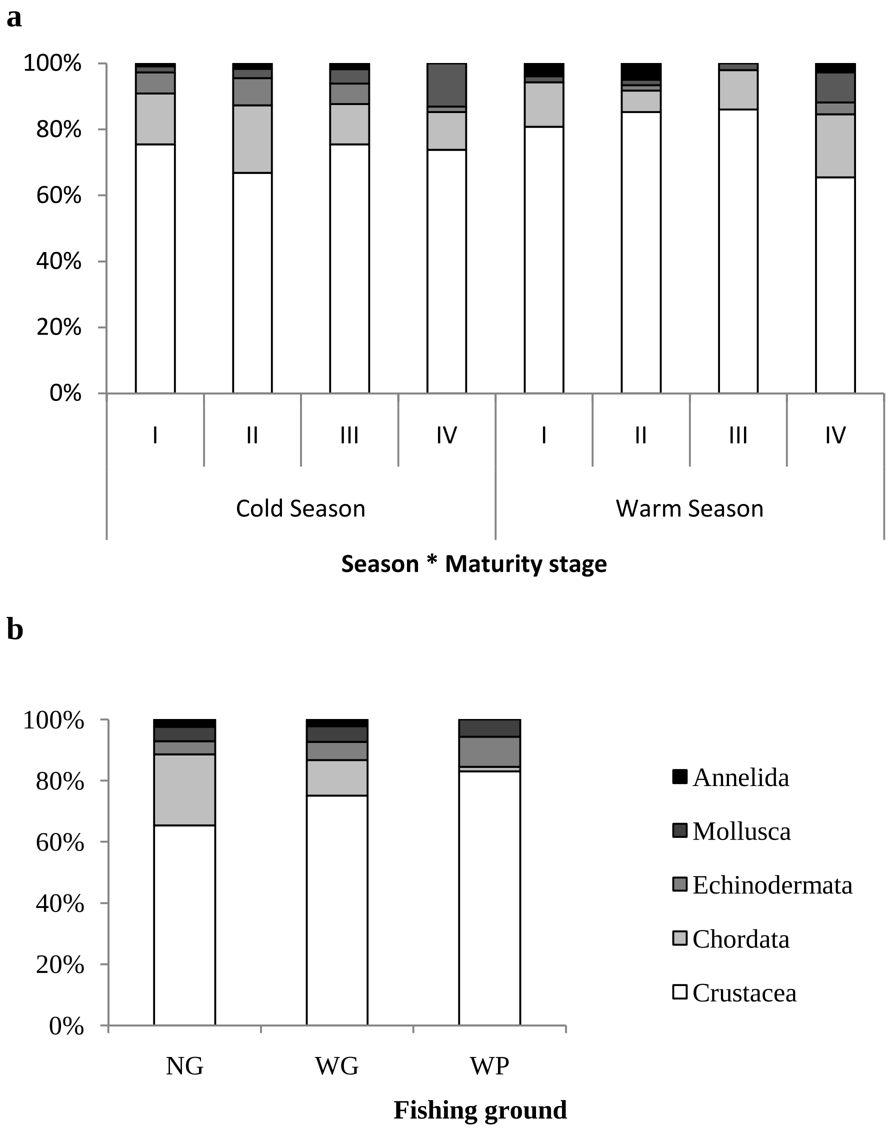


Figure 5.2. Diet composition of Eledone cirrhosa according to the significant variables highlighted by Multinomial Logistic Regression (MLR) model: Season*Maturity stage (a), and Fishing ground (b).

The emptiness index (EMI) in this study showed a wide variability across months, which agrees with the results by Moriyasu (1981), who also found significantly higher EMI in the warm season (53.43% in average) than in the cold one (39.67%), as found in this study. EMI decreased with BW in our specimens. This result, together with the higher EMI values attained during the cold season when mean size of the population is larger and individuals are close to the reproductive season (Regueira et al., 2013), suggests that food intake in larger animals increases with maturation. This hypothesis seems to be supported by the fact that gonadal development mainly depends on the energy intake from food (Regueira et al., 2013) and also because one of the major effects given by the model was the maturity stage. Nevertheless, as no clear pattern was inferred from EMI monthly evolution, significantly higher values of EMI reached by larger animals could indicate that larger individuals caught in net tows were less prone to regurgitate food than smaller ones. On the other hand, because the duration of throws of trawlers in this zone lasted approximately 6 hours, it could also be that the vacuity of stomachs might be due to the digestion of food. Nevertheless, given that *E. cirrhosa* needs 16 hours to free its digestive tract at a temperature of 18º C (Boucher-Rodoni, 1975) and the water temperature at depths where the throws are carried out is approximately 12ºC, this possibility does not appear to be a likely explanation. In any case, it is difficult to conduct dietary studies in the field as there are many variables that can introduce serious biases.

Concerning dietary regime analysis, although our final model was significantly better than the null model, Pseudo R-squared values indicated a poor fit. However, these types of metrics do not represent the amount of variance in the outcome variable accounted for by the predictor variables. Higher values indicate better fit, but they should be interpreted with caution. If a model has a very low likelihood, then the log of the likelihood will have a larger magnitude than the log of a more likely model, as occurred in the present study. As with most Chi-square based tests however, it is prone to inflation as sample size increases. Here we behold that the final model significantly improves the null model ($p < 0.001$), which indicates that our final model predicts significantly better, or more accurately, than the null model. In other words, when p-value is less than the established cut-off (generally 0.05) a good fit is obtained (Starkweather & Moske, 2011). Moreover, Pearson and Deviance goodness-of-fit tests, which compare observed and expected results, do not reject the null hypothesis and indicates a proper fitting.

Over the short life-span of cephalopods, a common pattern is for juveniles to prey on crustaceans and then switch to fish and other prey such as other cephalopods as they grow larger (e.g. Castro & Guerra, 1990; Nixon, 1987; Wangvoralak et al., 2011). Univariate analyses performed by Sifner and Vrgoc (2009) stated several factors that are involved in dietary changes in *E. moschata*, such as the onset of sexual maturity and size. According to their findings, larger animals have a more diversified diet, suggesting an increase of prey range and/or a change in nutritional requirements. Conversely, our model indicates that BW have not a significant effect on the probability of choosing a particular prey in *E. cirrhosa*, whereas the interaction of “Season” and “Maturity stage” significantly affect prey selection, in particular regarding mollusc ingestion. According to β parameters, mollusc ingestion attains higher proportion in mature animals. Since the vast majority of molluscs found in our samples were cephalopods, mainly *E. cirrhosa*, this result probably is largely referred to inter-cohort cannibalistic events. Dietary composition suggest that cannibalism is occasional in *E. cirrhosa* and, although it is unknown whether it takes place between animals of different sizes, observations in *O. vulgaris* (Hernández-Urcera et al., 2014) noted that prey/predator weight ratios range from 20% to 25% body weight. Our data indicate that cannibalistic episodes occurred in animals of various sizes during winter and early spring, both periods with an eventual abundance of juveniles (Regueira et al., 2014), so the presence of small animals could trigger this behaviour, as has been argued in the congeneric *E. moschata* (Sifner & Vrgoc, 2009). Despite the negative results in multicollinearity test, “Maturity stage” and “Season” are closely related to BW (Regueira et al., 2013), suggesting the underlying idea that this interaction comprises information concerning ontogenetic stage of the animals.

Similarly, our final model indicates that fishing ground significantly affected dietary regime, particularly the consumption of bony fish. Additionally, possible differences in the diet of *E. cirrhosa* between different geographic regions and seasons, as found in the present paper, had not been examined until now but these are common patterns for many cephalopods species (Hastie et al., 2009; Hatanaka, 1979). These dietary differences between regions may also be the result of a differential availability of different prey types among these areas. Thus, Fariña (1996) noted a significant decrease of fish biomass and a simultaneous rise of crustaceans with depth in the Galician continental shelf and upper slope. Nevertheless available information about the life cycle of the species leads to a more complex scenario, since *E. cirrhosa* average size significantly increases with latitude in the studied area (Regueira et al., 2013). On the other hand, a reproductive migration event to shallow waters is performed by this species during the warm season (Regueira et al., 2014) which could trigger an ontogenetic dietary change depending on maturation stage, as indicated by our model. In fact, fish contribution to *Eledone* sp. diet has been previously related to individual size of the octopuses (Ezzedine et al, 2012; Sifner & Vrgoc, 2009), since because of its high mobility, it constitutes a harder prey to catch than crustaceans. Hence, the variable "Fishing ground" in our model would account for geographic variability in the development of the animals as well as prey availability.

MLR is a powerful and useful tool for this type of study. Leite et al. (2009) succeeded in using this type of statistical analysis to study the influence of several factors on the feeding behaviour of *O. insularis*. Nevertheless, judging by their results, they limited themselves to presenting the first part of the regression, which measures the main effects (the impact of the independent variables on the dependent variable in a global view) without indicating which category it specifically affects. In our case, however, we attempted to bring biological sense to the values of β (Table 5.3), which indicate the relative (to the reference group) direction and intensity of the effect of every effect of each independent variable.

Acknowledgments

This research was supported by the project "Impacto do ambiente sobre o polbo *Eledone cirrhosa* no sistema de afloramento costeiro das augas de Galicia" (INCITE08PXIB402074PR), funded by Xunta de Galicia. Marcos Regueira was supported by a FCT grant (SFRH / BD / 51038 / 2010). We thank to Mª.T. Fernández and M. E. Garci for their help in the dissection of specimens and samples obtaining and also to M. Cueto for her help in genetic extraction.

References

Álvarez, I., Gomez-Gesteira, M., Castro, M.d., Lorenzo, M.N., Crespo, A. J. C. & Dias, J.M., 2010. Comparative analysis of upwelling influence between the western and northern coast of the Iberian Peninsula. Continental Shelf Research. 31:388-399.

Anderson, R.C., Wood, J.B. & Mather, J.A., 2008. *Octopus vulgaris* in the Caribbean is a specializing generalist. Marine Ecology Progress Series 371: 199−202.

Auteri, R., Manini, P. & Volpi, C. 1988. Regime alimentare di *Eledone cirrhosa* (Lamarck, 1798) e *Sepia orbignyana* (Férrussac in Orbigny, 1826) (Mollusca, Cephalopoda) nel Tirreno Settentrionale. Quaderni del Museo di Storia Naturale di Livorno 9: 67-73.

Bakun, A. & Nelson, C.S., 1991. The seasonal cycle of wind-stress curl in subtropical eastern boundary current regions. Journal of Physical Oceanography 21, 1815–1834.

Bashmachnikov, I., Neves, F., Nascimento, Â., Medeiros, J., Ambar, I., Dias, J. & Carton X., 2015. Temperature–salinity distribution in the northeastern Atlantic from ship and Argo vertical casts. Ocean Science 11, 215–236.

Belcari, P., Sartor, P., Jereb, P., Lefkaditou, E., Pierce, G.J., Piatkowski, U., Borges, T. & Allcock, A.L. 2015. *Eledone cirrhosa*. In: Jereb P., A.L. Allcock, E. Lefkaditou, U. Piatkowski, L. Hastie& G. J. Pierce (eds), Cephalopod biology and fisheries in Europe: II. Species Accounts, ICES Cooperative Report 325: 30-41.

Belsley, D.A., 2004. Conditioning Diagnostics. In: John Wiley & Sons I (ed). Encyclopedia of Statistical Sciences. doi:10.1002/0471667196.ess0275.pub2.

Bouvier, E.L., 1940. Faune de France. Décapodes marcheurs. Féderation Française des Sociétés de Sciences Naturelles, vol. 37. Paris.

Boucher-Rodoni, R., 1975. Vitesse de digestion chez les céphalopodes *Eledone cirrhosa* (Lamarck) et *Illex illecebrosus* (Lesueur). Cahiers de Biologie Marine 16:159-175.

Boyle, P.R., 1981. Methods for the aquarium maintenance of the commom octopus of British waters, *Eledone cirrhosa*. Laboratory Animals. 15: 327-331.

Boyle, P.R., 1983. *Eledone cirrhosa*. In: Boyle PR (ed) Cephalopod life cycles, vol 1: Species Accounts. Academic Press, London.

Boyle, P.R., 1986. A descriptive ecology of *Eledone cirrhosa* (Mollusca: Cephalopoda) in Scottish waters. Journal of the Marine Biological Association of the UK 66: 855-865.

Boyle, P.R., Knobloch, D., 1984. Reproductive maturity in fresh and acuarium-held *Eledone cirrhosa* (Cephalopoda: Octopoda). Journal of the Marine Biological Association of the UK 64: 581-585.

Boyle, P.R.& Rodhouse, P., 2005. Cephalopods: Ecology and Fisheries. Blackwell Science Ltd. Oxford UK.

Boyle, P.R., Grisley, M.S. & Robertson, G., 1986. Crustacea in the diet of *Eledone cirrhosa* (Mollusca: Cephalopoda) determined by serological methods. Journal of the Marine Biological Association of the UK 66:867-879.

Busch, W.-D.N., Brown, B.L. & Mayer, G.F. (Eds). 2003. Strategic Guidance for Implementing an Ecosystem-based Approach to Fisheries Management. United States Department of Commerce, National Oceanic and Atmospheric Administration, NMFS, Silver Spring, MD 62p.

Castro, B.G. & Guerra, A., 1990. The diet of *Sepia officinalis* (Linnaeus,1758) and *Sepia elegans* (Cephalopoda, Sepioidea) from the Ria de Vigo (NW Spain). Scientia Marina 54:375-388.

Deagle, B.E., Jarman, S.N., Pemberton, D. & Gales, N.J., 2005. Genetic Screening for Prey in the Gut Contents from a Giant Squid (*Architeuthis sp.*). Journal of Heredity 96:1-7.

Ezzeddine, S., Rjeibi M. & Chemmam, B., 2012. Regime alimentaire d´*Eledone cirrhosa* (Cephalopoda, Octopoda) des côtes nord et est Tunisiennes. Bulletin de l'Institut National des Sciences et Technologies de la Mer de Salammbô 39:25-33.

FAO, 2014. The State of World Fisheries and aquaculture. Opportunities and challenges. Rome. 223 pp.

Fariña, A.C., 1996. Megafauna de la plataforma continental y talud superior de Galicia. Biología de la cigala, *Nephrops norvegicus*. Universidad de La Coruña.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates.Molecular Marine Biology and Biotechnology 3: 294-299.

Gallagher, S.R. & Desjardins, P.R., 2006. Quantitation of DNA and RNA with absorption and fluorescence spectroscopy. Current Protocols in Molecular Biology 76: 3D:A.3D.1–A.3D.21.

Gislason, H., Sinclair, M., Sainsbury, K. & O'Boyle, R. 2000. Symposium overview: incorporating ecosystem objectives within fisheries management. ICES Journal of Marine Science 57: 468-475.

González-Gurriarán, E. & Méndez, M., 1986. Crustáceos decápodos das costas de Galicia. I. Brachyura. Cuadernos da Área de Ciencias Biolóxicas, Seminario de Estudos Galegos, Vol. 2. O Castro-Sada, A Coruña.

Guerra, A., 1992. Mollusca, Cephalopoda. In: *Fauna Ibérica*. Vol. 1 Ramos, M. A. *et al* (Eds.).Museo Nacional de Ciencias Naturales. CSIC. Madrid.

Hastie, L.C., Pierce, G.J., Wang, J., Bruno, I., Moreno, A. Piatkowski, U. & Robin, J.P., 2009. Cephalopods in the North-Eastern Atlantic: Species, Biogeography, Ecology, Exploitation and Conservation. Oceanography and Marine Biology, An Annual Review 47:111-190.

Hatanaka, H., 1979. Studies on the fisheries biology of common octopus off the northwest coast of Africa. Bulletin of the Far Seas Fisheries Research Laboratory 17:13-124.

Hernández-Urcera J., Garci, M.E., Roura, A., González, A.F., Cabanellas-Reboredo,M., Morales-Nin, B. & Guerra, A. 2014. Cannibalistic Behavior of *Octopus vulgaris* in the wild. Journal of Comparative Psychology 128:427-430.

Hureau, J.C., 1970. Biologie compareé de quelques poissons antarctiques (Nototheniidae). Bulletin de l'Institut océanographique de Monaco 68:1-50.

ICES WGHMM, 2013. Report of the Working Group on the Assessment of Southern Shelf Stocks of Hake, Monk and Megrim. ICES CM 2013/ACOH:11A.

Iglesias, J., Fuentes, L. & Villanueva, R., (Eds.). Cephalopod Culture. Springer, Netherlands.

Inejih, C. A., 2000. Dynamique spatio-temporelle et biologie du poulpe (*Octopus vulgaris*) dans les eaux mauritaniennes: modélisation de l'abondance et aménagement des pêcheries. Thése de Doctorat. Université de Bretagne Occidentale. 251 pp.

Leite, T.S., Haimovici, M. & Mather, J., 2009. *Octopus insularis* (Octopodidae), evidences of a specialized predator and a time-minimizing hunter. Marine Biology 156:2355-2367.

Mangold, K., Boletzky, S. v. & Frösch, D., 1971. Reproductive biology and embryonic development of *Eledonecirrhosa* (Cephalopoda: Octopoda). Marine Biology 8: 109-117.

Mather, J.A., 1991. Foraging, feeding and prey remains in middens of juvenile *Octopus vulgaris* (Mollusca: Cephalopoda). Journal of Zoology 224:27-39.

Moriyasu, M., 1981. Biologie des pêches de céphalopodes benthiques. Application aux eledones, *Eledone cirrhosa*(Lam., 1798) du golfe du Lion. Thèse Doctorat, 3ème cycle, Université des Sciences et Techniques du Languedoc, Montpellier, France.

Moriyasu, M., 1984. Egg clusters observed in the stomach contents of spawning females of *Eledone cirrhosa* (Lamarck) (Cephalopoda: Octopoda) from the North-western Mediterranean Sea. Venus; the Japanese Journal of Malacology 43:189-192.

Navarro, J.C., Monroig, Ó. & Sykes, A.V., 2014. Nutrition as a Key Factor for Cephalopod Aquaculture. In: Iglesias, J., Fuentes, L., Villanueva, R., (Eds.) Cephalopod Culture. Springer, Netherlands. 77-95 pp.

Nixon, M., 1987. Cephalopod diets. In: Cephalopod life cycles vol II. Academic press, pp 201-219.

Perrier, R., 1954. La faune de la France illustrée. Arachnides et Crustacés. Ed. Delagrave, Paris.

Pierce, G. J., Valavanis, V.D., Guerra, A., Jereb, P., Orsi-Relini, L., Bellido, J.M., Katara, I., Piatkowski, U., Pereira, J., Balguerias, E., Sobrino, I., Lefkaditou, E., Wang, J., Santurtun, M., Boyle, P.R., Hastie, L.C., MacLeod, C.D., Smith, J.M.,Viana, M., González A.F. & Zuur, A.F. 2008. A review of cephalopod-environment interactions in European Seas. Hydrobiologia 612:49-70.

Pikitch, E.K., Santora, C., Babcock, E.A., Bakun, A., Bonfil, R., Conover, D.O., Dayton, P., Doukakis, P., Fluharty, D., Heneman, B., Houde, E.D., Link, J., Livingston, P.A., Mangel, M., McAllister, M.K., Pope, J. & Sainsbury, K.J., 2004. Ecosystem-Based Fishery Management. Science 305: 346-347.

Pinkas, L., Olipham, M.S. & Iversor, I.L.K., 1971. Food habits of albacore, bluefin tuna and bonito in California waters. Fisheries Bulletin 152: 1-105.

Regueira, M., González, A.F., Guerra, A. & Soares, A., 2013. Reproductive traits of horned octopus *Eledone cirrhosa* in Atlantic Iberian waters. Journal of the Marine Biological Association of the UK 93:1641-1652. doi:10.1017/S0025315413000118.

Regueira, M., González, A.F. & Guerra, A., 2014. Habitat selection and population spreading of the horned octopus *Eledone cirrhosa* (Lamarck, 1798) in Galician waters (NW Atlantic). Fisheries Research 152:66-73. doi:http://dx.doi.org/10.1016/j.fishres.2013.09.003.

Roura, A., González, A.F., Redd, K. & Guerra, A., 2012. Molecular prey identification in wild *Octopus vulgaris* paralarvae. Marine Biology 159:1335-1345. doi:10.1007/s00227-012-1914-9.

Sánchez, P., 1981. Regime alimentare d'*Eledone cirrhosa* (Lamarck, 1798) (Mollusca, Cephalopoda) dans la Mer Catalane. Rapport Commission International Mer Mediterranee 27:209-212.

Schwab, J.A., 2002. Multinomial logistic regression: Basic relationships and complete problems. http://www.utexas.edu/courses/schwab/sw388r7/SolvingProblems/.

Serrano, A., Sánchez, F., Punzón, A., Velasco, F. & Olaso, I., 2011. Deep sea megafaunal assemblages off the northern Iberian slope related to environmental factors. Scientia Marina 75:425-437.

Sifner, S. K. & Vrgoc, N., 2009. Diet and feeding of the musky octopus, *Eledone moschata*, in the northern Adriatic sea. Journal of the Marine Biological Association of the UK 89:413-419.

Starkweather, J. & Moske, A.K., 2011. Multinomial Logistic Regression. http://www.unt.edu/rss/class/Jon/Benchmarks/MLR_JDS_Aug2011.pdf.

Symondson, W.O.C., 2002. Molecular identification of prey in predator diets. Molecular Ecology 11: 627-641.

Tamura, K., Stecher, G., Peterson, D., FilipskiA. & Kumar, S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30: 2725-2729.

Tuset, V.M., Lombarte, A. & Assis, C.A., 2008. Otolith atlas for the western Mediterranean, north and central eastern Atlantic. Scientia Marina. 72: 7-198.

Wangvoralak, S., Hastie, L.C. & Pierce, G.J., 2011. Temporal and ontogenetic variation in the diet of squid (*Loligo forbesii* Streenstrup) in Scottish waters. Hydrobiologia 670:223-240.

Watt, J., Pierce, G. J. & Boyle, P. R., 1997. Guide to the identification of north sea fish using premaxillae and vertebrae. ICES Cooperative Research Report. 220, 231 pp.

Wooster, W.S., Bakun, A. & Mclain, D.R., 1976. The seasonal upwelling cycle along the eastern boundary ofthe North Atlantic. Journal of Marine Research 34: 131–141.

Zariquiey A.,R., 1968. Crustáceos decápodos Ibéricos. Investigacion Pesquera. Vol. 32. Barcelona.

CHAPTER 6. Age and Growth

This chapter is published as Regueira M., González A.F. and Guerra Á., 2015. Determination of age and growth of the horned octopus Eledone cirrhosa *(Cephalopoda: Octopoda) using stylet increment analysis. Scientia Marina. 79(1):71-78.*

Determination of age and growth of the horned octopus *Eledone cirrhosa* (Cephalopoda: Octopoda) using stylet increment analysis

Summary

Age and growth of the horned octopus *Eledone cirrhosa* are estimated for the first time by stylet increment analysis (SIA). Specimens were collected between March 2009 and March 2010 from the commercial trawl fishery operating off north-western Iberian coasts. A pool of stylets, representing all size classes, were defined by means of modal progression analysis performed separately for each sex in northern and western fishing grounds over a total of 2220 individuals (306 males and 595 females from the northern fishing ground; and 379 males and 940 females from the western fishing ground). In total, 122 stylets, belonging to individuals with total weight ranging from 67 to 950 g, were successfully analyzed. Age of the specimens was estimated assuming a daily deposition rate of growth increments within the stylet. Estimated age of the specimens varied from 106 to 516 days. Differential longevity between sexes was observed, with females reaching a life span of 17 months while males attained just 14 months, although the possibility that this difference might be even greater is discussed. Based on estimated age, hatching date was back- calculated, concluding that hatching occurs throughout the year, with a peak during winter. Relative instantaneous growth rates (G) estimated for 90 days-age classes, ranged from 0.03 to 2.17% of BW per day, depending on age. The results obtained are compared with the information available on this species.

Running title: Age and growth of *Eledone cirrhosa* using stylets

Keywords: Eledone cirrhosa, stylet increment analysis, age, life span, growth, NW Iberian peninsula.

Introduction

Age determination is fundamental to understand and properly estimate various parameters that determine the biology of cephalopod species, such as growth rate, population structure, longevity, mortality, productivity and recruitment process (Boyle and Rodhouse, 2005). Furthermore, our knowledge of these processes, as well as conditioning factors, is essential to the proper management of fisheries resources. Due to the high intrinsic growth variability and consequent mixing of individuals of different ages (micro-cohorts) within the same size range, cephalopod studies on age and growth based on length and body weight measures are not considered as accurate as those undertaken by using hard structures (Jackson, 1994). However, those methodologies based on the external morphology of specimens are still employed because they are low cost and easy to apply in most fisheries (Semmens *et al.*, 2004).

Direct ageing methods, based on reading growth increments in different hard structures, have been developed to determine age in cephalopods. Particularly in octopods, beaks have provided some positive results in reading growth increments of Octopus vulgaris paralarvae (Hernandez-Lopez et al., 2001) and adults (Canali et al., 2011; Perales-Raya et al., 2010). Stylet Increment Analysis (SIA) has been applied and daily growth increment deposition validated in Octopus vulgaris (Hermosilla et al., 2010), Octopus pallidus (Doubleday et al., 2006), Octopus maya (Rodríguez-Domínguez et al., 2013) and *Octopus* (cf) *tetricus* (Leporati and Hart, 2014).

The horned octopus *Eledone cirrhosa* (Lamarck, 1798) is a medium-size benthic octopod, widely distributed over the continental shelves of the northeastern Atlantic and Mediterranean Sea (Boyle, 1983). This species breeds throughout the year, although in the north-west Iberian Peninsula reproduction mostly occurs between late spring and early summer (Regueira *et al.*, 2013). The reproductive peak in the western Mediterranean, at a similar latitude to the above mentioned study, is similarly timed (Lefkaditou and Papaconstantinou, 1995; Mangold-Wirz, 1963; Moriyasu, 1988). However, studies performed in Scotland indicate that egg laying occurs later, between July and September (Boyle and Knobloch, 1983), suggesting that latitude, through day-night duration, is a important parameter influencing sexual maturation (Regueira *et al.*, 2013).

Hatching season for this species in northern Europe has been supposed to happen mainly between October and December, about three or four months later than sexual maturation peak (Boyle, 1983). Little is known about early life stages of this species. Given the size of eggs (about 7 mm), considered as intermediate compared with those of other octopuses, hatchlings are certainly planktonic, but probably only for a matter of days, during which they remain near the bottom (Boyle, 1983). Account taken of all the above, a 20-month lifecycle has been postulated for this species in north European waters (Boyle and Knobloch, 1982), although this is unverified due to the lack of studies focusing on age and population structure of the species.

E. cirrhosa landings in Galician commercial harbors show a marked seasonality, typically associated with cephalopods, which have a short life-span and rapid population turnover (Sánchez and Martín, 1993). Captures amount also showed an increasing trend throughout the last decade (Regueira et al., 2014). Nevertheless, the biology of this octopus is still poorly studied in this region.

The main objective of the present study is to estimate age and growth of the horned octopus *Eledone cirrhosa* during their juvenile and adult stages using growth increments in stylets and obtain new insights into the lifecycle of this species in north-western Iberian Peninsula waters, such as life span and hatching season.

Material and Methods

From March 2009 to March 2010 samples of commercially caught horned octopus *E. cirrhosa* were collected monthly in three Galician (north-western Iberian Peninsula) fishing harbors: Burela (north) and Ribeira or Bueu (west). This species is caught as a by-catch in the multi-species trawl fishery operating in this whole area. Vessels landing their captures in Burela operate on northern fishing grounds, while landings in Bueu and Ribeira come from western fishing grounds (Figure 6.1).

In total, 2220 individuals of *E. cirrhosa* (306 males and 595 females from the northern fishing ground; and 379 males and 940 females from the western fishing ground) were processed. Measurements of each specimen included body weight (BW), eviscerated body weight (EBW), and dorsal mantle length (DML). Lengths and weights were measured to the nearest 0.1 cm and the nearest 0.1 g, respectively. Since specimens were mostly intact, no bias in weight measures was expected. The macroscopic maturity scale of Inejih (2000) was adapted to assign a specific maturity stage to each individual. For males this scale was as follows: I: immature, II: maturing, III: pre-spawning, with some spermatophores in Needham's sac, and IV: mature, fully developed spermatophore, and for females: I: immature, II: maturing, III: pre-spawning, IV: mature and V: post-spawning. Stylets of thirty specimens of each sex and fishing ground were taken monthly and stored in 70 % ethanol until preparation.

In order to identify size classes (grouped in 5 mm DML) and structure sampling, a modal progression analysis (MPA) was performed separately for males and females from both northern and western fishing grounds using the Battacharya method (1967) and FISAT software. Subsequently, three individuals representing each size class, sex and fishing ground were taken for SIA.

Permanent stylet preparations were made following four steps according to Barratt and Allcock methodology (2010). First, transversal sections of stylet approximately 1-2 mm thick were obtained using a razor blade. Second, sections were dehydrated by successive immersions for one hour in 70%, 90% and absolute ethanol. Third, sections were embedded in low viscosity acrylic resin (LR White^{TM}, London Resin Company). Fourth, in order to achieve a better light transmission and therefore a clearer view of the growth rings, the samples were ground and polished on both sides.

Samples were observed under a transmitted light microscope connected to an image analysis device equipped with LAS v1.4software. Stylet sections were photographed at 400x magnification under full light power, with the condenser adjusted to optimize brightness and contrast. A series of pictures were taken of each stylet section, covering its entire surface. Each complete image comprised from 4 to 54 partial pictures.

The stylet sections consisted of a striped-concentric surface surrounding a core, where each growth increment appears as a double band, comprising an optically translucent ring and a dark one (Figure 6.2). Each pair of bands was considered as one growth increment and, therefore, as a daily time indicator in the life of *E. cirrhosa*.

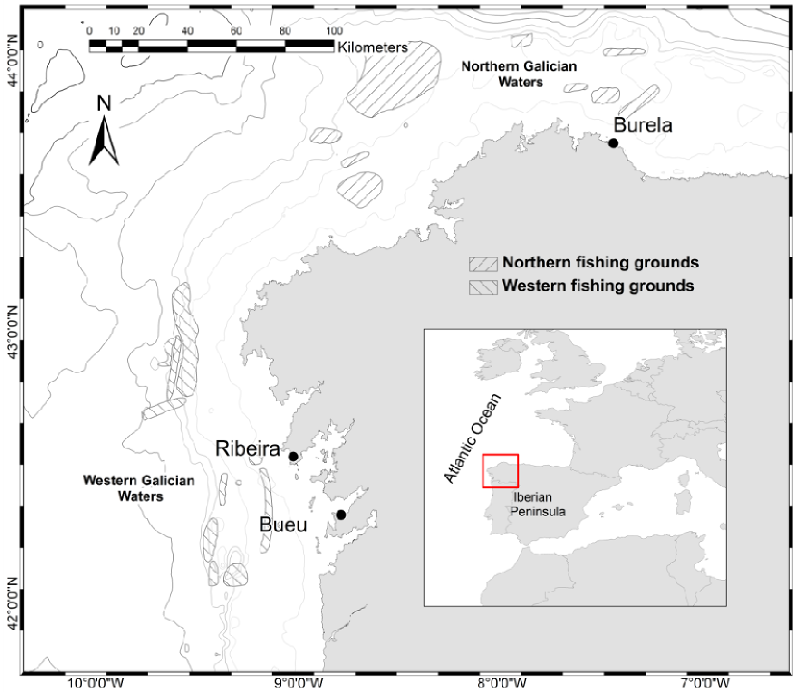


Figure 6.1. Location of the fishing ports where the *Eledone cirrhosa* specimens were between March 2009 and March 2010. Trawling fishing grounds catalogued in each zone are indicated.

In total, 264 stylets were prepared and photographed, 123 of which were considered unreadable, due to focusing or sample quality deficiencies. Two non-consecutive counts of growth increment were carried out in the remaining 141 stylets, from outer to inner zone. When the difference between counts exceeded 10% (Doubleday et al., 2006; Hermosilla et al., 2010) an extra count was undertaken. After eliminate samples with differences greater than 10%, 122 samples were accepted. These stylets represented 27 females and 39 males from the western fishing ground, and 33 females and 23 males from the northern fishing ground.

A preliminary observation of the photos taken from these stylet sections showed that the core area of the stylet was useless for reading, as found in other similar studies (e.g. Doubleday et al. 2006). Thus, Core Area (CoA, mm^2) and Counted Area (CA, mm^2) of each stylet were measured from pictures. The number of increments in the CoA was estimated by extrapolation following the increment deposition rate found in CA. This deposition rate for each stylet was obtained by fitting the potential relationship between CA and the number of counted increments. After logarithmic transformation, a t-test was preformed to compare both the slope and the intercept in growth increment deposition rate between males and females (Zar, 1984).

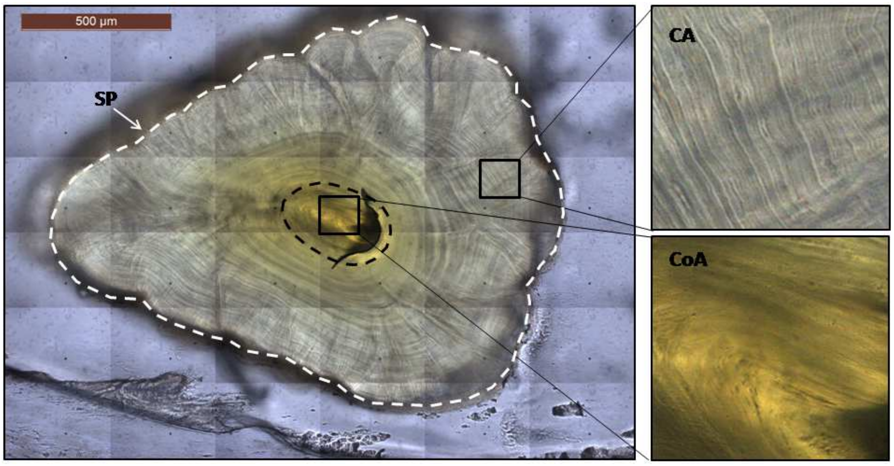


Figure 6.2. Composite image of an *Eledone cirrhosa* stylet preparation, showing the counted area (CA, the surface between dashed lines), in which growth rings are discernible, and the core area (CoA, the area within the dashed black line), where the growth rings appear obliterated. The number of growth rings in CoA was extrapolated following the deposition rate found in CA. Stylet perimeter (SP) is indicated by the white dashed line.

Based on previous studies on age validation by SIA performed in three octopus species (Doubleday et al., 2006; Hermosilla et al., 2010; Leporati and Hart, 2014) a daily deposition rate of growth increments in the stylet of *E. cirrhosa* was assumed. Thus, age of the specimens was calculated by adding the estimated growth rings in the CoA and those already counted in the CA and hatching dates were back calculated from capture date. Estimated age was then compared between sexes and fishing ground and was also related to BW, EBW and DML of the specimens.

Instantaneous relative growth rate (G, % BW d^{-1}) was calculated for each 90-day age class in order to obtain growth trends during different ontogenic periods, using the equations given by Forsythe and Van Heukelem (1987):

$$G = \frac{LnW_2 - LnW_1}{t_2 - t_1} x100,$$

where W_2 is the BW at age t_2 and W_1 is BW at age t_1. To estimate G, maximum and minimum BW for each 90 day age class were used.

Given that assumptions of normality and homocedasticity were not fulfilled, Spearman-rank correlation (r_s) was used to evaluate covariation between numerical variables and differences between categorical predictors were tested using non-parametric Kruskal–Wallis test, for multiple comparisons, and Mann–Whitney for paired comparisons, adjusting alpha by the Bonferroni approach (α_t) with the purpose of controlling Type I error across tests (Zar, 1984). All data were treated with the statistical software STATISTICA 6.0.

Results

Modal Progression Analysis (MPA)

Modal progression analysis was performed separately for females and males of western and northern fishing grounds off Galician coasts. Dorsal mantle length (DML) of the 2220 considered specimens ranged from 57 to 158 mm for males and from 45 to 180 mm for females. Body weight (BW) ranged between 40 and 634 g for the males and from 15 to 1090 g for females. Table 6.1 shows the monthly size classes per sex and fishing ground determined by SIA. During the period between March 2009 and March 2010, six size classes for females on both fishing grounds were identified. Conversely, males showed only five size classes in this period. A maximum of three simultaneous size classes per group in one month were detected (Table 6.1). Size classes were followed over time for as much as nine months.

Stylet Increment Analysis (SIA)

The 122 stylets finally accepted for counting growth increments comprised 60 females (BW: 67 to 950 g, DML: 73 to 174 mm), and 62 males (BW: 68 to 498 g, DML: 64 to 140 mm).

Stylet sections varied widely in shape, from nearly circular to much more irregular. No clear zonation, apart from the readable and unreadable areas, was observed. The readable area constituted between 90 and 99.7 % of the total stylet section area. In other words, assuming a circular shaped stylet section, the unreadable zone was equivalent to the internal area defined by a radius of between 5.5% and 31.62% of the total radius.

No significant ($p>0.05$) differences were found between both slopes and intercept in growth increment deposition rates between females and males, which indicate that sex has no effect on deposition rates. Therefore, a general equation was estimated from combined data of both sexes. The equation relating the readable area of the stylet and the counted increments (CI) in this area was: $CI = 241.7*CA^{0.507}$; $R^2 = 0.756$ (Figure 6.3). Based on this equation, growth rings in the CoA were estimated.

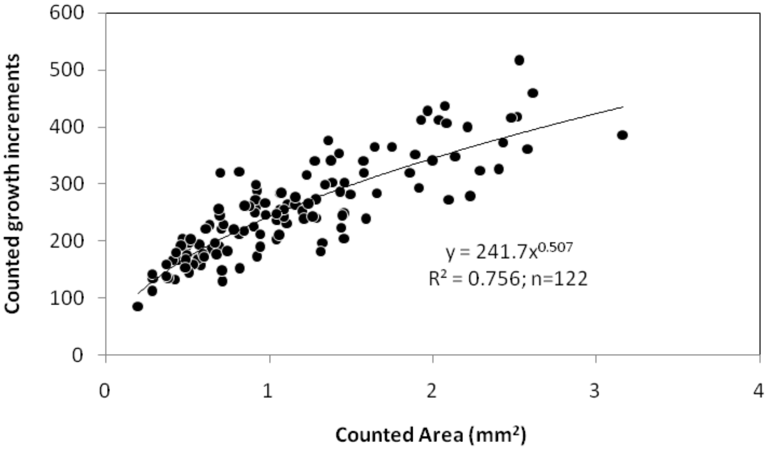


Figure 6.3. Relationship between counted area (CA) and number of counted growth increments in the stylet section of *Eledone cirrhosa*.

Estimated age

Significant differences ($p< \alpha_t$) in the estimated age between males and females were found in both fishing grounds; however, it remained constant for each sex regardless of the origin ($p>\alpha_t$). Overall estimated age varied from 160 to 516 days for females, and from 106 to 420 days for males. Estimated age in the northern fishing ground ranged from 167 to 516 d (mean ± S.D.: 339.9 ± 76 d) for females and from 180 to 381 (mean ± S.D.: 270.8 ± 58 d) for males. In the western fishing ground, estimated age ranged from 160 to 512 days (mean ± S.D.: 315.4 ± 96 d) for females, and from 106 to 420 days (mean ± S.D.: 239.6 ± 71 d) for males.

The estimated age of the specimens was significantly correlated with all measurements considered (BW, EBW and DML) for pooled data and also when analyzing sexes and fishing grounds separately ($p < 0.05$). The best predictor of age for females was BW, with a r_s of 0.77 and 0.62 for the western and northern fishing ground, respectively. However, the best predictor proved to be DML ($r_s = 0.64$) for males on the north coast, and EBW ($r_s = 0.72$) for males of the west coast. Figure 6.4 illustrates the relationships between estimated age and BW and DML.

Age of mature males ranged from 186 to 381 days (mean ± S.D.: 292.8±74) on the northern fishing grounds while it varied between 170 and 286 days (mean ± S.D.: 243.7±40) in western waters. With the exception of one specimen with an estimated age of 167 days, the estimated age of mature females ranged between 310 and 453 days (mean ± S.D.:348.8±55) in the northern fishing grounds and from 386 to 450 days (mean ± S.D.:418±45) on the western fishing grounds (Figure 6.5). No post-spawning females were found.

Hatching date vs. Size class

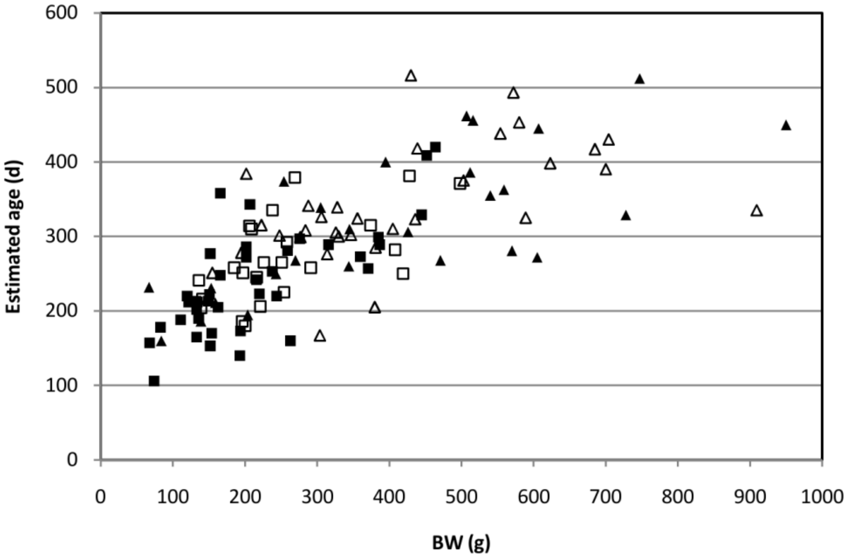
Hatching dates of individuals captured between March 2009 and March 2010 ranged from December 2007 to October 2009 and hatching occurred throughout the year, with a peak in winter (Figure 6.6).

Size classes determined by MPA partially match with predicted hatching date (Figure 6.7), however they do not predict age groups, since hatching dates of individuals belonging to different size classes do overlap. Thus, paired comparisons of hatching date between consecutive size classes did not show significant differences ($p>\alpha_t$). Differences in estimated age between individuals assigned to a particular size class at the same month ranged from 6 to 286 days, with a mean of 78.1±64 days (Mean ±S.D.). Regarding to areas and sex, difference between western females differed was 84.4±76 days, while in northern females was 92.8±84 days. This latter group achieved the largest differences in estimated age within a particular cohort, up to 286 days. Concerning males, the difference was 66.9±37 days, and 60.6±38 days, for the western and northern coasts, respectively.

Table 6.1. Summary of size classes detected per month on each fishing ground for males and females of *Eledone cirrhosa*. Mean dorsal mantle length (DML; mm ± S.E.) for each class is indicated.

Northern Fishing ground	**Mar-09**	**Apr-2009**	**May-09**	**Jun-09**	**Aug-2009**	**Sep-09**	**Oct-09**	**Nov-09**	**Dec-2009**	**Feb-10**	**Mar-10**	**Size class**
Females	124.3±4.8	141±3	140.8±5.1	-	-	-	-	-	-	-	-	1
	98.9±8.5	117.3±3.4	118.4±5.9	138.9±11	-	-	-	-	-	-	-	2
	75±4.2	96.7±6.7	95±4.2	116.9±3.4	117.46±6.1	136.2±15.7	132.3±2.3	163.4±2.5	-	-	-	3
	-	-	-	-	94±5.8	105.9±9.8	113.3±8.6	127.8±10.7	139.7±8.9	143.7±12.1	161.5±5.6	4
	-	-	-	-	-	-	-	95±2.5	114.2±8.5	126±2.5	141±7.6	5
	-	-	-	-	-	-	-	-	-	103.3±9.6	117.5±6	6
Males	95±3	118.3±7	108.6±3.6	117.5±4.6	-	-	-	-	-	-	-	1
	-	95±3.4	87.4±5	102.5±4.2	109.9±6.8	-	122.7±6.3	-	-	-	-	2
	-	-	-	-	97.5±5.2	103.7±6.1	106.11±3.2	-	-	-	-	3
	-	-	-	-	-	-	-	114±5.2	-	132.5±7.8	-	4
	-	-	-	-	-	-	-	-	-	105±3.7	-	5
Western Fishing ground												
Females	127.5±7.3	114.3±6.1	-	-	-	-	-	-	-	-	-	1
	99.6±6.6	103.4±4.6	105.4±5.9	112.4±8.8	116.8±9.7	-	-	-	-	-	-	2
	76.2±7.7	79±8.2	80.2±5.6	84.4±8	96.4±8.3	109.7±7.8	140.6±3.7	153.2±5.6	-	-	-	3
	-	-	-	-	64.7±5.5	85±5.5	125±4.6	133.6±6.1	-	-	-	4
	-	-	-	-	-	-	-	112.5±7.8	113.7±10	119.5±10.4	129.3±7.1	5
	-	-	-	-	-	-	-	-	94±9.9	94.1±7.7	96.2±5.3	6
Males	103.9±7.48	98.9±4.7	-	-	-	-	-	-	-	-	-	1
	85.5±3.5	75.7±12.2	95.96±5.8	97.5±8.3	103.9±6.1	112.9±5.8	123.4±4.35	129.03±6	-	-	-	2
	-	-	-	83.29±6.7	90±3.8	96±5	-	112.5±9.3	-	-	-	3
	-	-	-	-	-	-	75±6.7	87.5±6	-	-	102.9±8.5	4
	-	-	-	-	-	-	-	-	-	79.9±6	82.5±6	5

A



B

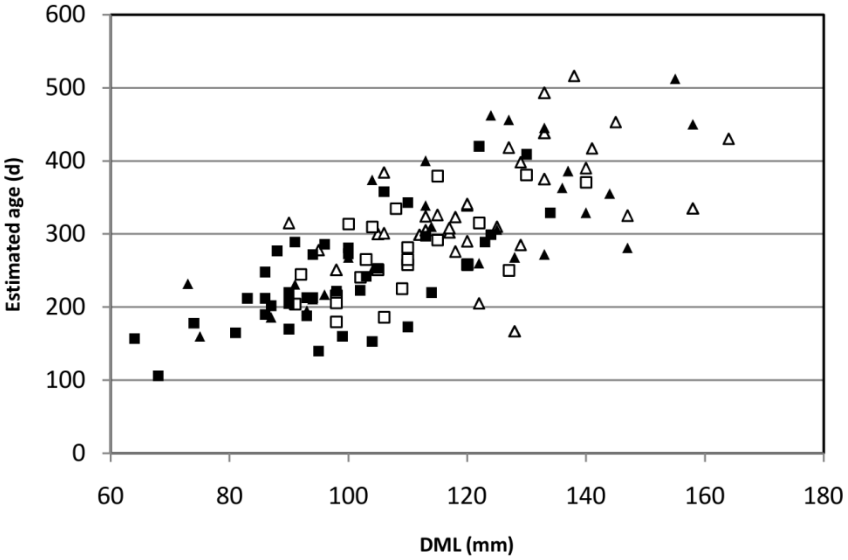


Figure 6.4. Scatterplots showing estimated age (d) of each *Eledone cirrhosa* specimen versus BW in grams (A) and DML in millimetres (B). ■ = West coast males (n=39), □ = North coast males (n=23), ▲ = West coast females (n=27) and △ = North coast females (n=33).

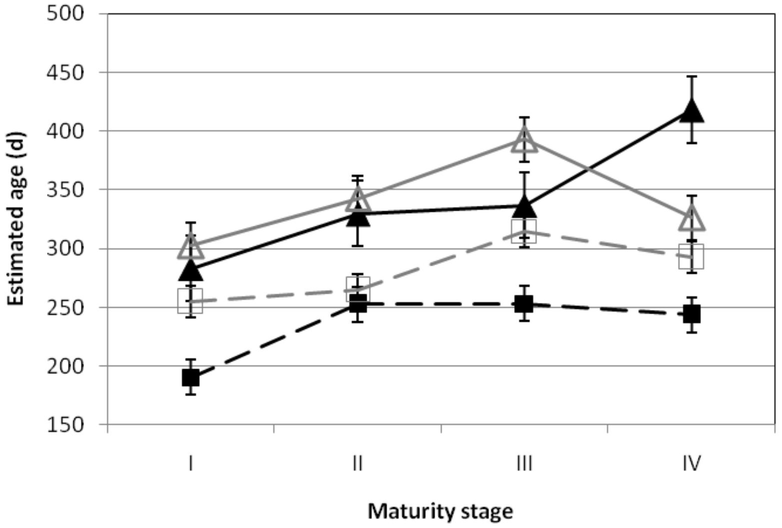


Figure 6.5. Mean age per maturation stage by sex and fishing ground. ■ = West coast males (n=39; black dotted trend line), □ = North coast males (n=23; grey dotted trend line), ▲ = West coast females (n=27; Continuous black line) and △ = North coast females (n=33; Continuous grey line). No post-spawning females were found.

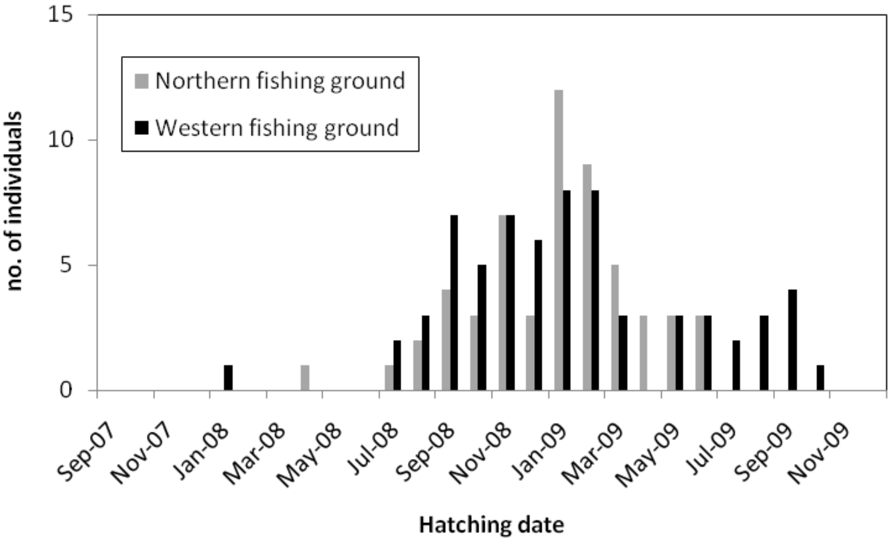


Figure 6.6. Hatching date histogram, resulting from back-calculation according to capture data and estimated age of the 122 successfully aged *Eledone cirrhosa* specimens.

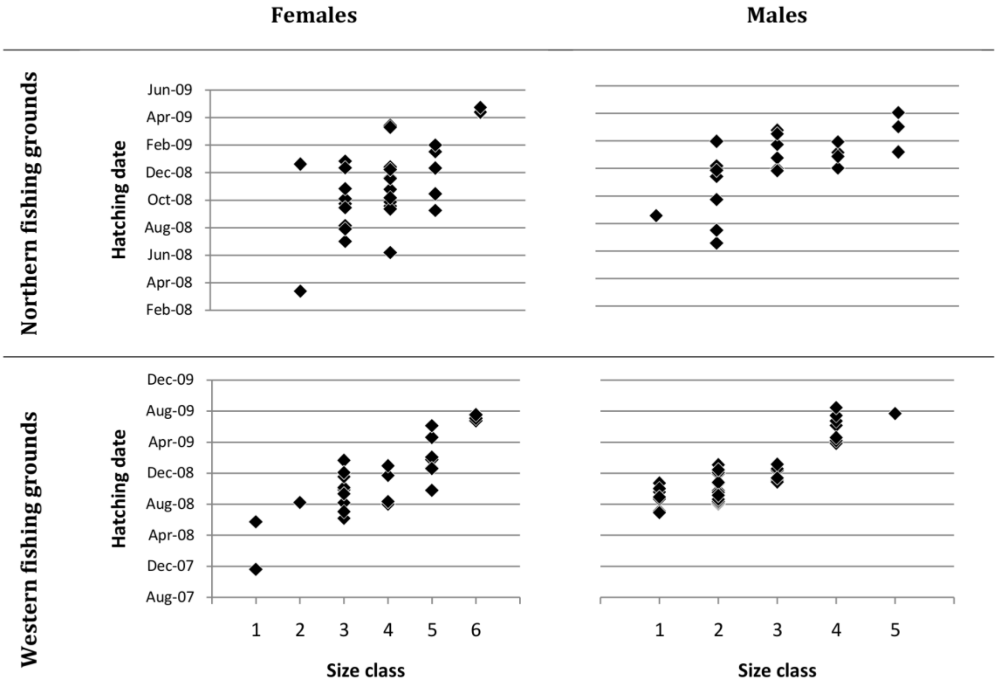


Figure 6.7. The graphics illustrates the application of MPA as a proxy for defining age classes in *Eledone cirrhosa*. Specimens of each sex and fishing ground are displayed separately, according to their estimated hatching date and the size class they belong to. Size classes were previously defined by MPA and followed in time, so specimens of a particular size class would be expected to have hatched at about the same date. Nevertheless, range of hatching date of the specimens belonging to consecutive size-classes does largely overlap in many cases.

Growth

Relative instantaneous growth rates (G) generally decreased with age (Figure 6.8). Values of G varied between age classes from 0.03 to 2.17% of BW per day (Mean± SD: 1.07±0.54). Male G varied from 0.03 to 1.5% (Mean± SD: 0.97±0.51); female G varied from 0.33 to 2.17% (Mean± SD: 1.15±0.58).

Although the Student t-test does not reject the null hypothesis of homogeneity of slopes ($p>0.05$), young individuals from the western fishing ground do have higher growth rates than those from the north according to the calculated G. However, their growth rate subsequently decreases more sharply, reaching lower values of G.

The adjusted slope is similar in both sexes within each sampled area; however, the value of the intercept differs between sexes, and males attained a lower G than corresponding-age females.

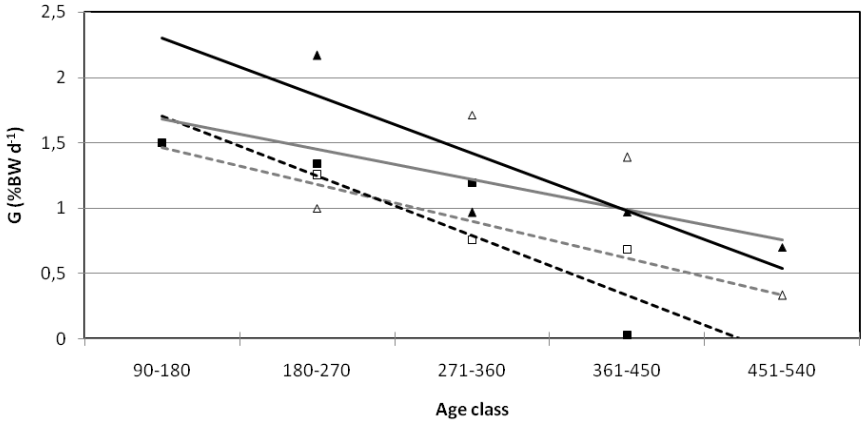


Figure 6.8. *Eledone cirrhosa* instantaneous relative growth rate (G) in BW (% BW d-1) and trending lines per 90 day age classes. ■ = West coast males (black dotted trend line), □ = North coast males (grey dotted trend line), ▲ = West coast females (Continuous black line) and △ = North coast females (Continuous grey line).

Discussion

Our results show that *E. cirrhosa* hatches throughout the year in north western Iberian waters, with a peak in January. The comparison of our data with the reproductive cycle in this area, where the maximum gonadosomatic index of females peaks in June (Regueira *et al.*, 2013), indicates that the period between maturity peak and egg hatching takes about six months. Considering that embryonic development at 16°C takes about 110 days in this species (Mangold *et al.*, 1971) and since sea bottom temperature on the north-western Iberian continental shelf ranges between 11°C and 14°C (Gago *et al.*, 2011), this longer time period should be expected. Experiments in culture indicate that females of *E. cirrhosa* may copulate several weeks before egg laying begins and can preserve sperm for up to six weeks (Mangold *et al.*, 1971). Therefore, although the reproductive peak occurs in June, egg laying may be delayed until the summer and hatching until December.

A significant differential longevity between sexes was observed in *E. cirrhosa*, with females living longer than males. A general tendency for this fact in octopods cannot be established based on previous studies. Thus, neither Hernandez-Lopez *et al.* (2001) nor Canali *et al.* (2011) found significant differences in lifespan between sexes in *O. vulgaris*. Conversely, Leporati *et al.* (2008) found significant differences in age by reading *O. pallidus* stylets, although males were significantly longer-lived than females. This lack of uniformity in the published results could suggest inconsistencies in sampling method, rather than physiological differences between species. These inconsistencies may be due to a different catchability between sexes during different life stages, as might result from females of *E. cirrhosa* migrating to shallow water to spawn (Regueira *et al.*, 2014); during this reproductive migration, they may be more vulnerable to fishing, while a proportion of males remain in deeper areas, outside the fishing grounds worked by trawlers. This could be the reason why females seem to be much more abundant than males in this fishery. On the other hand, females of *E. cirrhosa* lays its eggs on rocky substrate in a similar way to *O. vulgaris*, and certainly not on open smooth muddy or sandy bottoms, and likely broods the eggs (Mangold *et al.*, 1971). If this is so, females might not be captured during this period, while males could be captured throughout their life. The absence of post-spawning females in this study supports this fact. Given the above, lifespan difference between sexes would be of up to almost eight months. Combining this with the results on age estimate obtained in this study (maximum age of 517 days), the life span of female *E. cirrhosa* would be around two years, agreeing with the hypothesis of Boyle (1986) for Scottish populations.

According to minimum estimated ages, recruitment to the fishery of *E. cirrhosa* in northwestern Iberian waters would occur when animals reach 3-4 months old. This also agrees with fishery catch pattern of the species in north-western Galician waters, in which a peak of captures corresponding to new recruits takes place around May (Regueira *et al.*, 2014). Thus, the concluded chronological pattern matches with that proposed by Mangold (1971) when analyzing embryonic development of the species.

Despite the significant correlation found between size and age, in view of our results, MPA seems not to provide an adequate vision of age-structure in *E. cirrhosa* populations. The application of MPA as a tool for inferring age structure has been rejected before on cephalopod populations, mainly due to the high intrinsic variability in growth rates (Forsythe and Van Heukelem, 1987) and also because neither length nor weight are good indicators of the age of a soft-bodied animal (Forsythe, 1984). Although some authors have suggested its validity if it can correct the observation bias introduced by the size-selective fishing gear (Keyl *et al.*, 2011), our results support the former hypothesis, given the high size diversity between same-age specimens. This individual diversity is also illustrated by the wide variety of mathematical models used to define the growth of cephalopods, due to the unsuitability of a single model to take account of the wide variability in sizes of individuals belonging to a unique age-class (Semmens *et al.*, 2004). Given this lack of consensus, it seems likely that cephalopods present different types of growth in different stages of development, so the growth rates set out in this work are only valid for the benthic life-stage and cannot be extrapolate to embryonic and planktonic stages.

Based on size at first maturity (DML50%) estimated for this species in north-western Iberian coasts (Regueira *et al.*, 2013) and DML-Age relationships in this paper, females would achieve maturity at around one year old, while males would do so at approximately 8 or 9 months.

Estimated growth rates are similar results to those estimated for other octopods in the wild, such as *Octopus mimus* (0.09-1.25%) (Cortez *et al.*, 1999). According to experiments in the laboratory, octopuses generally demonstrate two phase growth: an initial rapid exponential - phase and a slower power growth phase (Semmens *et al.*, 2004); therefore, a progressive moderation of growth rate would be expected, or at least in young individuals, it should remain stable. Given the limited amount of successfully aged individuals, and although the observed trends are plausible, further analysis will be needed to accurately determine such changes in growth rates as well as for embrionary and planktonic phases. Moreover, as in other cephalopods, different micro-cohorts may have different growth rates depending on the time of hatching (Pierce and Guerra, 1994). The lack of precise catch location data could explain part of the variability in our results, as well as reported segregated distribution by sex and size (Regueira *et al.*, 2014), since individuals caught in different areas may be subjected to diverse abiotic and biotic conditions affecting growth processes (Pierce *et al.*, 2008).

The method for sample preparation described by Barrat and Allcock (2010) and applied herein provided durable samples, which were kept throughout the process of experimentation and photography. This proves the suitability of the method for age studies in *Eledone cirrhosa*.

In the future, the results of the present study will need further validation. Not only because the deposition rate of growth rings could have a different periodicity to a day, but also because stylet formation and growth processes remain unknown during embryonic development and early life stages and, as observed in other structures such as statoliths (González *et al.*, 2010), the central area of the stylet may undergo compaction during early growth. That would make it harder to estimate the number of rings in this area and could bias the results on estimated age. However, the concordance between this interpretation of the population structure, by mean of age estimates, with the reproductive cycle described for the species and the pattern of catches supports the validity of what is concluded in this paper.

Acknowledgements

This research was supported by the project "Impacto do ambiente sobre o polbo *Eledone cirrhosa* no sistema de afloramento costeiro das augas de Galicia" (INCITE08PXIB402074PR), funded by Xunta de Galicia. Marcos Regueira was supported by a FCT grant. We thank M.E. Garci and M.T. Fernández for their help in the dissection of specimens and collection of stylets.

References

Barratt, I. M., Allcock, A. L. 2010. Ageing octopods from stylets: development of a technique for permanent preparations. ICES J. Mar. Sci. 67, 1452–1457.

Bhattacharya, C. G. 1967. A simple method of resolution of a distribution into gaussian components. Biometrics. 23, 115-135.

Boyle, P. R. , Rodhouse, P. G. 2005. Cephalopods. Ecology and Fisheries, Blackwell Science Ltd, Oxford, 464 pp.

Boyle, P. R. 1983. *Eledone cirrhosa*. In P.R. Boyle (ed.) Cephalopod life cycles. Vol 1: Species Accounts, 365-386. Academic Press. London.

Boyle, P. R. 1986. A descriptive ecology of *Eledone cirrhosa* (Mollusca: Cephalopoda) in Scottish waters. J. Mar. Biol. Ass. U.K. 66, 855-865.

Boyle, P. R., Knobloch, D. 1982. On growth of the Octopus *Eledone cirrhosa*. J. Mar. Biol. Ass. U.K. 62, 277-296.

Boyle, P. R., Knobloch, D. 1983. The female reproductive cycle of the octopus, *Eledone cirrhosa*. J. Mar. Biol. Ass. U.K. 63, 71-83.

Canali, E., Ponte, G., Belcari, P., Rocha, F., Fiorito, G. 2011. Evaluating age in *Octopus vulgaris*: estimation, validation and seasonal differences. Mar. Ecol. Prog. Ser. 441, 141–149.

Cortez, T., González, Á. F., Guerra, Á. 1999. Growth of *Octopus mimus* (Cephalopoda, Octopodidae) in wild populations. Fish. Res. 42, 31-39.

Doubleday, Z., Semmens, J., Pecl, G., Jackson, G. 2006. Assessing the validity of stylets as ageing tools on *Octopus pallidus*. J. Exp. Mar. Biol. Ecol. 338 (1), 35-42.

Forsythe, J. W. 1984. *Octopus joubini* (Mollusca: Cephalopoda): a detailed study of growth through the full life cycle in a closed seawater system. J. Zool. 202, 393-417.

Forsythe, J. W. Van Heukelem, W. F., 1987. Growth. in P.R. Boyle (ed.) Cephalopod Life Cycles Vol 2: Comparative Reviews, 135-156. Academic Press. London.

Gago, J., Cabanas, J. M., Casas, G., Miranda, A. 2011. Thermohaline measurements in the continental shelf zone of the NW Iberian Peninsula, 1994–2006. Clim. Res. 48, 219-229.

González, Á. F., Otero, J., Pierce, G. J., Guerra, Á. 2010. Age, growth, and mortality of *Loligo vulgaris* wild paralarvae: implications for understanding of the life cycle and longevity. ICES J. Mar. Sci. 67, 1119-1127.

Hermosilla, C. A., Rocha, F., Fiorito, G., González, Á. F., Guerra, Á. 2010. Age validation in common octopus *Octopus vulgaris* using stylet increment analysis. ICES J. Mar. Sci. 67, 1458–1463.

Hernandez-Lopez, J. L., Castro-Hernandez, J. J., Hernandez-Garcia, V. 2001. Age determined from the daily deposition of concentric rings on common octopus (*Octopus vulgaris*) beaks. Fish. Bull. 99, 679-684.

Inejih, C. A. 2000. Dynamique spatio-temporelle et biologie du poulpe (*Octopus vulgaris*) dans les eaux mauritaniennes: modélisation de l'abondance et aménagement des pêcheries. Thése de Doctorat. Université de Bretagne Occidentale. 251 pp.

Jackson, G. D. 1994. Application and future potential of statolith increment analysis in squids and sepioids. Can. J. Fish. Aquat. Sci. 51, 2612-2625.

Keyl, F., Argüelles, J., Tafur, R. 2011. Interannual variability in size structure, age, and growth of jumbo squid (*Dosidicus gigas*) assessed by modal progression analysis. ICES J. Mar. Sci. 68, 507–518.

Lefkaditou, E., Papaconstantinou, C. 1995. Distribution, growth and maturity of *Eledone cirrhosa* (Cephalopoda:Octopoda) in the Thracian Sea (Eastern Mediterranean). Rapp. Comm. int. Mer Médit, 34.

Leporati, S. C., Semmens, J. M., Pecl, G. T. 2008. Determining the age and growth of wild octopus using stylet increment analysis. Mar. Ecol. Prog. Ser. 367, 213-222.

Leporati, S. C., Hart, A. M. 2014. Stylet weight as a proxy for age in a merobenthic octopus population. Fish. Res. 161, 235-243.

Mangold-Wirz, K. 1963. Biologie des Céphalopodes bentiques et nectoniques de la Mer Catalane. Vie Milieu, 13(Suppl.). 285 pp.

Mangold, K., Boletzky, S. v., Frösch, D. 1971. Reproductive biology and embryonic development of *Eledone cirrhosa* (Cephalopoda: Octopoda). Mar. Biol. 8, 109-117.

Moriyasu, M. 1988. Analyse de la maturation sexuelle d´*Eledone cirrosa* (Cephalopoda : Octopoda) du golfe du Lion. Aquat. Living. Resour. 1, 59-65.

Perales-Raya, C., Bartolomé, A., M. Teresa García-Santamaría, Pascual-Alayón, P., Almansa, E. 2010. Age estimation obtained from analysis of octopus (*Octopus vulgaris* Cuvier, 1797) beaks: Improvements and comparisons. Fish. Res. 106, 171-176.

Pierce, G. J. Guerra, A., 1994. Stock assessment methods used for cephalopod fisheries. Fish. Res. 21, 255-285.

Pierce, G. J., Valavanis, V. D., Guerra, A., Jereb, P., Orsi-Relini, L., Bellido, J. M., Katara, I., Piatkowski, U., Pereira, J., Balguerias, E., Sobrino, I., Lefkaditou, E., Wang, J., Santurtun, M., Boyle, P. R., Hastie, L.C., MacLeod, C. D., Smith, J. M., Viana, M., González, A. F., Zuur, A. F. 2008. A review of cephalopod-environment interactions in European Seas. Hydrobiologia. 612, 49-70.

Regueira, M., González, A. F., Guerra, A. 2014. Habitat selection and population spreading of the horned octopus *Eledone cirrhosa* (Lamarck, 1798) in Galician waters (NW Atlantic). Fish. Res. 152, 66-73.

Regueira, M., González, A. F., Guerra, Á., Soares, A. 2013. Reproductive traits of horned octopus *Eledone cirrhosa* in Atlantic Iberian waters. J. Mar. Biol. Ass. U.K. 93, 1641-1652.

Rodríguez-Domínguez, A., Rosas, C., Méndez-Loeza, I., Markaida, U. 2013. Validation of growth increments in stylets, beaks and lenses as ageing tools in *Octopus maya*. J. Exp. Mar. Biol. Ecol. 449, 194-199.

Sánchez, P., Martín, P. 1993. Population dynamics of the exploited cephalopod species of the catalan sea (NW Mediterranean). Sci. Mar. 57, 153-159.

Semmens, J. M., Pecl, G. T., Villanueva, R., Jouffre, D., Sobrino, I., Wood, J. B., Rigby, P. R. 2004. Understanding octopus growth: patterns, variability and physiology. Mar. Freshwater Res. 55, 367-377.

Zar, J. H. 1984. Biostatistical analysis. Englewood Cliffs, NJ: Prentice-Hall. 718 pp.

CHAPTER 7. Experience in aquarium

Some records on the behaviour of the horned octopus *Eledone cirrhosa* in captivity

Abstract

A total of sixteen individuals of horned octopus *Eledone cirrhosa* (Lamarck, 1798) were caught using creels by a vessel of the small-scale fishery operating in the Ría of Vigo (NW Spain). Captures took place on the west side of the Cíes Islands (Galician National Park of the Atlantic Islands) in March 2013 between 60 and 70 meters depth. All the octopuses were females, ranging 355-950 g in body weight. Animals were kept alive and subsequently transferred to open seawater system. Observations under confined conditions resulted in several behavioural events, summarized as follows: i) visual attack sequence was described and found to be similar to that exhibited by *Sepia* sp., which involves three main phases: attention, positioning and seizure, ii) after a time of confinement, females spontaneously start to spawn in the artificial dens arranged inside the tanks, although eggs were unviable, and iii) a burrowing behaviour, similar to that exhibited by *Octopus vulgaris,* was observed for the first time. These observations are discussed and compared with previous literature.

Keywords: *Eledone cirrhosa*, hunting, spontaneous spawning, fake spawn, burrowing.

Introduction

The horned octopus *Eledone cirrhosa* (Lamarck 1798) is from both landing significance and its wide distribution range, the second octopod species in commercial importance in European waters, only after *Octopus vulgaris* (Cuvier 1797). *E. cirrhosa* is a ubiquitous species in the Mediterranean Sea and its geographic range extends beyond the Strait of Gibraltar, over the continental shelf of Western Europe and Northern Africa, from the coast of Morocco, southwards, and through the Atlantic side of the Iberian Peninsula and the British Isles, until Norway and Iceland coasts (Boyle 1983; Guerra 1992). It is a benthic species inhabiting sandy and muddy bottoms (Boyle 1983) of the slope and continental shelf, between 50 and 300 meters depth in the Mediterranean basin, being more frequent between 60 and 120 m (Belcari et al. 2002). This depth pattern distribution seems to be similar to that found in the southern parts of its Atlantic range, although in Scotland and northwards this species also inhabits the coastal zones (Boyle 1983).

Because of the habitat it occupies, *Eledone cirrhosa* is a by-catch for the commercial trawling fleet and, therefore, obtaining live animals for captive study is particularly difficult. So that, the little we know about its reproductive behaviour has been indirectly inferred from analyses carried out with specimens coming from commercial fisheries and some few observations in confinement (Iglesias et al. 2014), mainly carried out by Boyle and collaborators in the Zoological Laboratory of the University of Aberdeen in the eighties (see Boyle, 1983 for a review).

Based on sex ratio bias found in different studies and its variation, this species had been proposed to perform a segregated distribution according to sex and maturity (Mangold-Wirz 1963; Mangold et al. 1971). Moreover, mature females move inshore for egg laying (Boyle 1997) at the beginning of the spawning season. This seasonal migration takes place at different time throughout its geographic range (Boyle et al. 1988). Specifically in NW Iberian peninsula it takes place mainly from May to June (Regueira et al. 2013). This aspect was recently confirmed on the basis of standardized captures in North-western Iberian waters (Regueira et al. 2014).

Available information on spawning can be summarized as follows: i) egg laying duration is variable, and lasts from 8 days to nearly 1 month depending on temperature

(See Villanueva & Norman, 2008 for a review); ii) egg strings usually contain eggs at different developmental stages (Villanueva and Norman 2008); and iii) embryonic development duration varies between 3–4 months at 14–18°C (Mangold et al. 1971).

In this paper we present a series of observations on visual attack sequence, spawning and burrowing behaviour of the horned octopus *E. cirrhosa*, based on the observation of animals under aquarium conditions. Given the difficulties in obtaining viable octopuses and the subsequently limited number of experiences of this kind performed with this particular species, we considered the described herein as relevant information for the knowledge of this species.

Material and Methods

A total of sixteen individuals of the octopus *E. cirrhosa* were obtained from a vessel of the small-scale fishery operating around the Cíes Islands, within the limits of the *Galician Atlantic Islands Maritime-Terrestrial National Park*, in NW Spain. Three of these animals were caught in March the 13th, and the remainders six days after. Depth of capture ranged from 60-70 m. The octopuses were transported alive to the facilities of the Institute of Marine Research in Vigo (IIM- CSIC). All the animals were weighed and sexed; being determined as females, ranging 355-950 g in body weight (BW).

Octopuses were kept in four circular, flat bottomed, fibreglass tanks; measuring 135x50 cm (diameter and height). Tanks were equipped with seawater open circuit supply. Raw water temperature in three of the tanks maintained at 15° C and in the remaining one was cooled to 10 °C by with an electric cooler. Photoperiod was set to the natural conditions with of a clock timer, which controlled light periods.

In order to minimize potential aggressive behaviours, four individuals of similar size were placed in each tank. Four PVC pipe sections and/or plastic pots, were placed inside each tank, thus providing shelters to the animals. Escape of the octopuses from the tanks was prevented by covering them with a plastic mesh.

The animals were feed *ad libitum* with live crabs *Carcinus maenas* (about 6-7 cm carapace width) and alternatively, when crabs were not available, with pieced fresh horse mackerel *Trachurus trachurus*.

Behaviour of the animals was filmed by a GoPro Hero 3 video camera.

Results

Aquarium-held animals in the facilities at IIM-CSIC quickly adapted to captivity conditions and started feeding three or four days after being introduced into the tanks. Although the animals were receptive to all kind of food, live crabs powerfully drew octopuses' attention, triggering hunting behaviour. Conversely, fresh fish was received with lower interest.

Most of the animals considered in this study feed early in the morning and/or at night, keeping mostly inactive during the daylight period, as expected for this species (Cobb et al. 1995).

Visual attack sequence

Hunting behaviour was found to show a consistent pattern. According to our observations, this behaviour is triggered by visually detection of its prey. Then the octopus changes its chromatic pattern from a finely grained mottle of yellow-red colour with white spots (Figure 7.1A) to an intense roughly grained reddish-colour while gently approaches (Figure 7.1-B). When the octopus gets a distance of about one time its own mantle length (Figure 7.1-C), it pounces on and catches the crab using its brachial crown-umbrella complex (Figure 7.1-D), while maintaining the intense reddish coloured chromatic pattern.

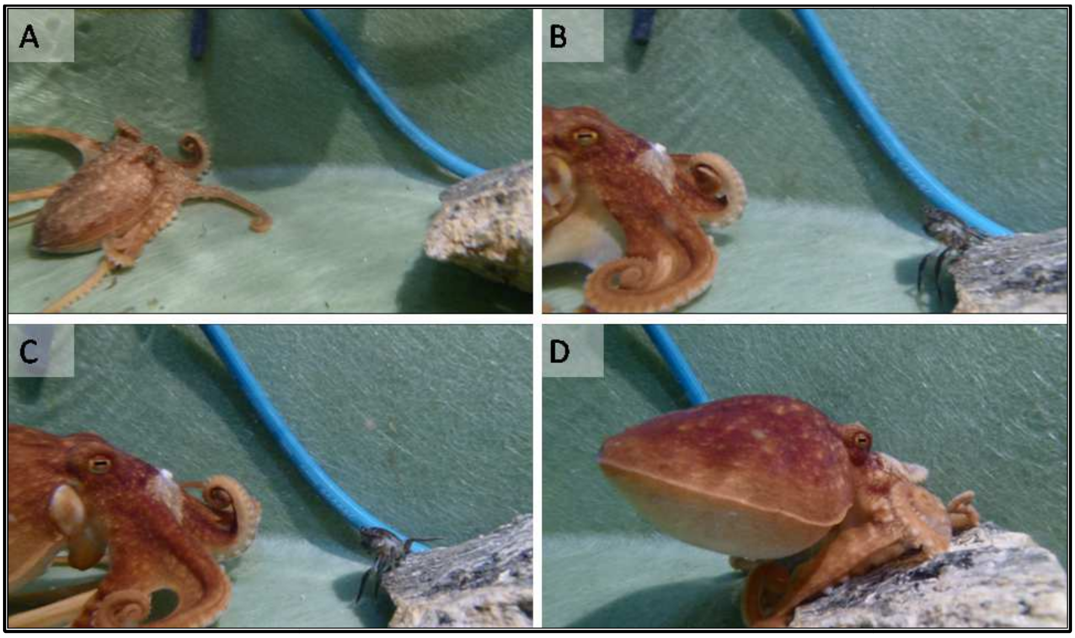


Figure 7.1. *Eledone cirrhosa* attack sequence to a crab. **A**: Resting individual, showing a mottled body pattern; **B**: The octopus establishes visual contact with the prey, triggering hunting behaviour. Then the octopus gently approaches to the prey. Note the changes in chromatic and body patterns; **C**: When a distance of about one time its own dorsal mantle length is reached, pounces on the crab; **D**: the octopus traps the crab using its brachial crown-umbrella complex.

Spawning

On the 27thApril, 2013 one of the females (725g) started attaching the egg strings on the ceilings and interior walls of a shelter (Figure 2). That specimen and her shelter were isolated in a 150 l glass aquarium, in order to minimize the stress potentially caused by the presence of other individuals in the nearby, as well as to facilitate its monitoring. Moreover, in this aquarium was arranged a soil of sand and different materials, like stones or shells, in order to recreate the natural environment. At once, and in order to follow up egg strings without disturbing the female, 5 egg strings were taken and placed in a small mesh cage. After 27 days of monitoring them, neither the isolated eggs nor the laid inside the shelter experienced any change, except for a slight darkening colour.

During the study, four additional females started laying eggs in fibreglass tanks, attaching the egg strings within shelters as the first spawning female did. Eventually, some isolated eggs were found attached to the walls of the tank, although due to that several females shared each tank, it was not possible to determine which female spawned them.

From the spawning event onward, all these spawning females gradually reduced their food ingestion rate until they stopped feeding.

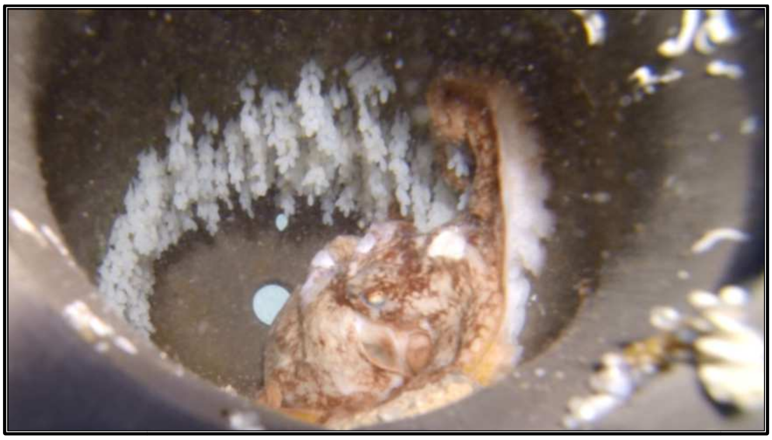


Figure 7.2. *Eledone cirrhosa* female inside a shelter. Egg strings were located on the ceiling. This animal showed severe skin lesions.

Burrowing behaviour

The first spawning female, placed in the individual tank, performed a burrowing behaviour, which can be summarized as follows: i) the spawning female always remained inside the shelter; ii) she blocked the entrance with stones and/or other different materials available from the adjacent environment (Figure 3), iii) the animal showed an increasingly physical degradation (e.g. skin ulcerative lesions, low ingestion rate, if any, etc.) during the "incubation" time (Figure 2); iv) the animal died on May 24th and during these 28 days 532 eggs were laid in 33 clusters, which represents an average of 16.1 eggs per cluster.

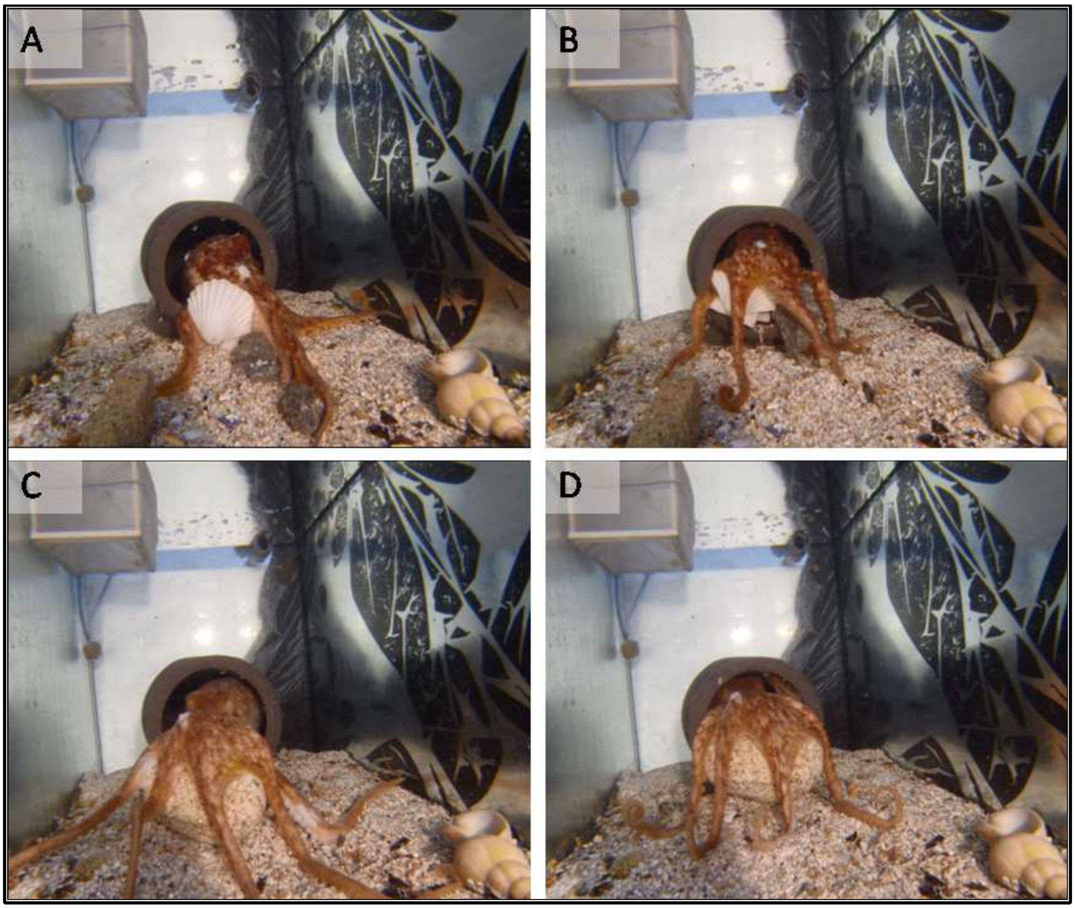


Figure 7.3. Sequence of images showing the burrowing behaviour of *Eledone cirrhosa*. The female collected several available materials from their environment sealing the entrance of the shelter.

Discussion

Although *Eledone cirrhosa* has been reported to be primarily caught with bottom trawl and to a lesser extent with seines (Jereb et al. 2014), as far as we know, this is the first record of the capture of this species with creels in Atlantic Iberian waters, where it is regularly captured by bottom trawl in the continental shelf (between 200-400 m depth). Nevertheless, the animals referred herein were caught in much more shallower waters (60-70 m). According to fishermen this event is not rare during several weeks a year in spring, when large individuals appear within baited creels in relatively shallow waters. This information agrees with the seasonal offshore-inshore reproductive migration described for the species in this particular area (Regueira et al. 2014), and is also consistent with the fact that all captured individuals were large females.

Visual attack sequences occurred in our tanks involved different phases, similar to those described by Messenger (1968) for cuttlefishes, which ambush their prey through three phases: attention, positioning and seizure. This hunting behaviour we observed can be considered a kind of stalking (Hanlon and Messenger 1996). The intense and rough, grained and red-coloured chromatic pattern displayed by *E. cirrhosa* while stalking and pouncing on the prey (Figure 1B, C and D) could seem contradictory with a stalking hunting strategy, opposite to the finely grained mottle of yellow-red colour, defined as "mottle" by Hanlon and Messenger (1996) for octopods and as "dressing-gown pattern" by Boyle and Dubas (1981) for *E. cirrhosa*, which usually display the animals at rest and undisturbed (Figure 1A). Nevertheless, red light attenuates rapidly with depth in the water and, therefore, a red organism would appear black at depth, rendering it invisible to their predators (McClintock et al. 2001), making this chromatic pattern suitable in the wild. That is why many mesopelagic and deep-sea benthic species are red-coloured (Johnsen 2005), as *E. cirrhosa* is. Thus, the reddish colour of this species constitutes an adaptation to the environment that inhabits and this stereotyped hunting behaviour increases its cryptic ability during pouncing. In consequence, we hypothesized that *E. cirrhosa* instantly adopts this chromatic pattern, becoming virtually invisible to its prey and, therefore conducting an stealth-ambush strategy in the deep clear soft bottoms which this species inhabits.

Both territoriality and aggressive behaviour against neighbours due to defence of adjacent surroundings or shelter home has been found in several octopod species (Aronson 1986; Cigliano 1993; Mather et al. 1985), which is frequently promoted by dominance hierarchies based on size (Mather 1980). In fact, cannibalism has been reported in this species (Guerra 1992, Regueira et al., 2007; Moriyasu 1981) and also in the common octopus (*O. vulgaris*) inhabiting nearby coastal areas (Hernández-Urcera et al. 2014). However, neither territorial behaviour nor hostility was observed among the animals sharing the same tank during this study. The lack of cannibalism could be due to the similar sizes of the specimens sharing each tank as well as keeping the animals fed *at libitum*. On the other hand, the occurrence of this type of behavior is really low and could be triggered only in very concrete circumstances of high abundance of juvenile individuals, as suggested by Regueira et al. (2017).

Our observations showed that five females spontaneously spawned in the tanks. Although no systematic attempt has been made to investigate the factors which may be influencing this event, it is known that aquarium conditions trigger gonadic ripeness (Boyle and Knobloch 1984). Therefore, since the individuals considered in this study were captured while migrating for spawning, it is not surprising that all of them were mature females. However the whole set of eggs was unviable, and it remains unclear whether the eggs were actually fertilized or not. So the underlying question is whether or not the females were previously mated and, therefore the eggs were fertilized before their catch. References on the spontaneous spawn in cephalopods under confined conditions have been scarcely published. The only reference concerning to a closely related species was provided by Valic (2005) for which he called “fake spawning”, in *E. moschata*. Wodinsky (1972) in *O. vulgaris* and Guerra (pers. obs.) in *Sepia officinalis* observed some individuals laying infertile eggs when brought into the aquarium and not mated there, although no explanation was offered.

Previous studies on *E. cirrhosa* in the laboratory suggested that this species deposits its eggs strings on rocky substrate in a similar way that *O. vulgaris*, and certainly not on open smooth muddy or sandy bottom (Mangold et al. 1971), but this behaviour has never been directly observed in this species. Although some of our animals deposited isolated egg in the walls of the tank, which can be considered an aberrant behaviour (Mangold et al. 1971), most of them laid the eggs strings within a shielded place. Thus, according to our observations, *E. cirrhosa* females spawn within a shelter, inside which they remain during eggs development, and block the entrance with stones and other materials they find nearby the burrow. This fact had never been reported before, so our observations confirm what, until now, was merely speculation. Moreover they perform an active care of the egg lying until their death, as many other benthic cephalopod species do (Hanlon and Messenger 1996).

Burying behaviour has been previously described for *E. cirrhosa* (Guerra et al. 2006). However, this behaviour was not observed in our case, even though a sandy bottom was placed on the glass aquarium of the first spawning female, suggesting that this behaviour is performed when a lack of available shelter occurs. Since it is known that *E. cirrhosa* is a typical soft-bottom species, this observation provides a plausible explanation about the reproductive migratory behaviour described for the species. We hypothesized that mature females might migrate to shallower waters in order to increase the availability of shielded places (crevices or caves in rocky substrates) suitable for spawning.

Acknowledgments

We thank to *Nuevo Cigala*'s crew for providing the specimens for experimentation. This research was supported by the proyect "Impacto do ambiente sobre o polbo *Eledone cirrhosa* no sistema de afloramento costeiro das augas de Galicia" (INCITE 08PXIB402074PR), funded by Xunta de Galicia. Marcos Regueira was supported by a FCT grant (SFRH/BD/51038/2010). We also thank the contributions of M.E. Garci, A. Roura and M. Bao.

References

Aronson, R.B., 1986. Life history and den ecology of *Octopus briareus* Robson in a marine lake. Journal of Experimental Marine Biology and Ecology 95, 37-56.

Belcari, P., Tserpes, G., González, M., Lefkaditou, E., B., M., Piccinetti, G.M., Souplet, A., 2002. Distribution and abundance of *Eledone cirrhosa* (Lamarck, 1798) and *E.moschata* (Lamarck, 1798) (Cephalopoda: Octopoda) in the Mediterranean Sea. Scientia Marina 66, 143-155.

Boyle, P.R., 1983. *Eledone cirrhosa*, in: Boyle, P.R. (Ed.), Cephalopod life cycles. Academic Press, pp. 365-386.

Boyle, P.R., 1997. *Eledone cirrhosa*: biology and fisheries in the Eastern Atlantic and Mediterranean, in: Lang, M.A., Hochberg, F.G. (Eds.), Proceedings of the workshop on the fishery and market potential of *Octopus* in California. Smithsonian Institution, Washington D.C., pp. 99-103.

Boyle, P.R., Dubas, F., 1981. Components of body pattern displays in the Octopus *Eledone cirrhosa* (Mollusca: Cephalopoda). Marine Behavior & Physiology 8, 135-148.

Boyle, P.R., Knobloch, D., 1984. Reproductive maturity in fresh and acuarium-held *Eledone cirrhosa* (Cephalopoda: Octopoda). Journal of the Marine Biological Association of the United Kingdom 64, 581-585.

Boyle, P.R., Mangold, K., Ngoile, M.A., 1988. Biological variation in *Eledone cirrhosa* (Cephalopoda: Octopoda): Simultaneous comparison of North Sea and Mediterranean populations. Malacologia 29, 77-87.

Cigliano, J.A., 1993. Dominance and den use in *Octopus bimaculoides*. Animal Behaviour 46.

Cobb, C.S., Pope, S.K., Williamson, R., 1995. Circadian rhythms to light-dark cycles in the lesser octopus, *Eledone cirrhosa*. Marine and Freshwater behaviour and physiology 26, 47-57.

Guerra, A., 1992. Mollusca, Cephalopoda. Fauna Ibérica. Museo Nacional de Ciencias Naturales. CSIC. Madrid. 1, 327 pp.

Guerra, A., Rocha, F., González, Á.F., González, J.L., 2006. First observation of sand-covering by the Lesser Octopus *Eledone cirrhosa*. Iberus 24, 27-31.

Hanlon, R.T., Messenger, J.B., 1996. Cephalopod Behaviour. Cambridge University Press.

Hernández-Urcera, J., Garci, M.E., Roura, A., González, A.F., Cabanellas-Reboredo, M., Morales-Nin, B., Guerra, A., 2014. Cannibalistic Behavior of *Octopus vulgaris* in the Wild. Journal of Comparative Psicology 128, 427-430.

Iglesias, J., Fuentes, L., Villanueva, R., 2014. Cephalopod culture, London.

Jereb, P., Roper, C.F.E., Norman, M.D., Finn, J.K., 2014. Cephalopods of the world. An annotated and illustrated catalogue of cephalopod species known to date. Volume 3. Octopods and Vampire Squids., Rome.

Johnsen, S., 2005. The Red and the Black: Bioluminescence and the Color of Animals in the Deep Sea. Integrative and Comparative Biology 45, 234-246.

Mangold-Wirz, K., 1963. Biologie des Céphalopodes bentiques et nectoniques de la Mer Catalane. Vie et Milieu., 285 pp.

Mangold, K., Boletzky, S.v., Frösch, D., 1971. Reproductive biology and embryonic development of *Eledone cirrhosa* (Cephalopoda: Octopoda). Marine Biology 8, 109-117.

Mather, J.A., 1980. Social organization and use of space by *Octopus joubini* in a semi-natural situation Bulletin of Marine Science 30, 848-857.

Mather, J.A., Resler, S., Cosgrove, J., 1985. Activity and movement patterns of *Octopus dofleini*. Marine Behavior & Physiology 11, 301-314.

McClintock, J.B., Baker, B.J., Steinberg, D.K., 2001. The Chemical Ecology of Invertebrate Meroplankton and Holoplankton, in: McClintock, J.B., Baker, B.J. (Eds.), Marine Chemical Ecology. CRC press.

Messenger, J.B., 1968. The visual attack of the cuttlefish, *Sepia officinalis*. Animal Behaviour 16, 342–357.

Moriyasu, M., 1981. Biologie des peches de céphalopodes benthiques. Applica-tion aux eledones, *Eledone cirrhosa* (Lam. 1798) du golfe du Lion. . Université des Sciences et Techniques du Langue-doc, France p. 225.

Regueira, M., González, A.F., Guerra, A., 2014. Habitat selection and population spreading of the horned octopus *Eledone cirrhosa* (Lamarck, 1798) in Galician waters (NW Atlantic). Fisheries Research 152, 66-73.

Regueira, M., González, A.F., Guerra, Á., Soares, A., 2013. Reproductive traits of horned octopus *Eledone cirrhosa* in Atlantic Iberian waters. Journal of the Marine Biological Association of the United Kingdom 93, 1641-1652.

Valic, D., 2005. "Fake" spawning of the musky octopus *Eledone moschata* (Lamarck, 1798) in experimental conditions. Ribarstvo 63 61-67.

Villanueva, R., Norman, M.D., 2008. Biology of the planktonic stages of benthic octopuses. Oceanography and Marine Biology: An Annual Review 46, 105-202.

Wodinsky, J., 1972. Breeding Season of Octopus vulgaris. Marine Biology 16, 59-63.

CHAPTER 8. Final conclussions

Reproduction

1. Size of *Eledone cirrhosa* inhabiting north-western Iberian waters increases with latitude, following the general pattern found in the whole distribution range of the species.
2. Significant different sizes were found between sexes.
3. The bottom trawl fishery operating in the area mainly catches females, which comprised 74.71% of the individuals sampled.
4. Significant differences in sex-ratio were found between Galician and Portuguese samples.
5. The spawning season of *Eledone cirrhosa* in north-west Iberian waters extends from March to September, with a peak in May-June.
6. The potential fecundity ranged from 547 to 6545 oocytes per ovary.
7. Mantle length in mature males was positively correlated with spermatophore length, but not with spermatophore number.
8. Size at first maturity increases with latitude in both sexes.
9. Digestive gland does not act as a storage organ for gonadic ripeness.

Distribution

10. According to surveys performed between June and November, higher abundances of *E. cirrhosa* occur in October-November and between 100 and 200 meters depth.
11. The migratory pattern of the species leads to higher abundances in coastal areas during early summer. This coastal abundance disappears in August-September, concentrating at the edge of the slope and spreading throughout the continental shelf in October-November.

Diet and feeding

12. The diet of the horned octopus *Eledone cirrhosa* in Atlantic Iberian waters is mainly based on crustaceans (70.6%), followed by fish (16.8%) and, in a lesser extent, by echinoderms (5.5%), molluscs (4.9%) and polychaeta (2.1%).
13. The main factors affecting prey ingestion are Maturity stage, Fishing ground and Season.

Age and growth

14. Longevity differed significantly between sexes, with females reaching a life span of 17 months while males attained only 14 months.
15. *E. cirrhosa* in North-western Iberian waters hatches throughout the year, with a peak in January.
16. Relative instantaneous growth rates, estimated for 90 day age classes, ranged from 0.03% to 2.17% of body weight per day, decreasing with age.

Behaviour

17. A three-phased hunting behaviour, similar to that exhibited by *Sepia* sp., was observed under confinement conditions.
18. Spontaneous spawning was observed under aquarium conditions, although the eggs were not viable.
19. Nesting behaviour under aquarium conditions was described for the first time in *E. cirrhosa*. During this period the animal remains inside the shelter and blocks the entrance with stones and/or other different materials available from the adjacent environment.